ACS SYMPOSIUM SERIES [illegible]

Flavors in Noncarbonated Beverages

EDITED BY

Neil C. Da Costa and Robert J. Cannon

Flavors in Noncarbonated Beverages

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.fw001

In Flavors in Noncarbonated Beverages; Da Costa, N., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.fw001

ACS SYMPOSIUM SERIES **1036**

Flavors in Noncarbonated Beverages

Neil C. Da Costa, Editor
International Flavors & Fragrances Inc.

Robert J. Cannon, Editor
International Flavors & Fragrances Inc.

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.fw001

Sponsored by the
ACS Division of Agricultural & Food Chemistry

American Chemical Society, Washington, DC

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.fw001

Library of Congress Cataloging-in-Publication Data

Flavors in noncarbonated beverages / [edited by] Neil C. Da Costa, Robert J. Cannon ; sponsored by the ACS Division of Agricultural & Food Chemistry.
p. cm. -- (ACS symposium series ; 1036)
Includes bibliographical references and index.
ISBN 978-0-8412-2551-0 (alk. paper)
1. Non-alcoholic beverages--Flavor and odor. 2. Fruit juices--Flavor and odor. 3. Energy drinks. 4. Tea. I. Da Costa, Neil C. II. Cannon, Robert J. III. American Chemical Society. Division of Agricultural and Food Chemistry.
TP620.F53 2010
663'.6--dc22
2010003338

The paper used in this publication meets the minimum requirements of American National Standard for Information Sciences—Permanence of Paper for Printed Library Materials, ANSI Z39.48n1984.

Copyright © 2010 American Chemical Society

Distributed by Oxford University Press

All Rights Reserved. Reprographic copying beyond that permitted by Sections 107 or 108 of the U.S. Copyright Act is allowed for internal use only, provided that a per-chapter fee of $40.25 plus $0.75 per page is paid to the Copyright Clearance Center, Inc., 222 Rosewood Drive, Danvers, MA 01923, USA. Republication or reproduction for sale of pages in this book is permitted only under license from ACS. Direct these and other permission requests to ACS Copyright Office, Publications Division, 1155 16th Street, N.W., Washington, DC 20036.

The citation of trade names and/or names of manufacturers in this publication is not to be construed as an endorsement or as approval by ACS of the commercial products or services referenced herein; nor should the mere reference herein to any drawing, specification, chemical process, or other data be regarded as a license or as a conveyance of any right or permission to the holder, reader, or any other person or corporation, to manufacture, reproduce, use, or sell any patented invention or copyrighted work that may in any way be related thereto. Registered names, trademarks, etc., used in this publication, even without specific indication thereof, are not to be considered unprotected by law.

PRINTED IN THE UNITED STATES OF AMERICA

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.fw001

Foreword

The ACS Symposium Series was first published in 1974 to provide a mechanism for publishing symposia quickly in book form. The purpose of the series is to publish timely, comprehensive books developed from the ACS sponsored symposia based on current scientific research. Occasionally, books are developed from symposia sponsored by other organizations when the topic is of keen interest to the chemistry audience.

Before agreeing to publish a book, the proposed table of contents is reviewed for appropriate and comprehensive coverage and for interest to the audience. Some papers may be excluded to better focus the book; others may be added to provide comprehensiveness. When appropriate, overview or introductory chapters are added. Drafts of chapters are peer-reviewed prior to final acceptance or rejection, and manuscripts are prepared in camera-ready format.

As a rule, only original research papers and original review papers are included in the volumes. Verbatim reproductions of previous published papers are not accepted.

ACS Books Department

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.pr001

Preface

This book is based upon the symposium entitled "Flavors in noncarbonated beverages" presented at the American Chemical Society (ACS) 236th National Meeting in Philadelphia, Pennsylvania on August 17-21, 2008. The symposium was sponsored by the ACS Division of Agriculture and Food Chemistry,

This book endeavors to capture the most important elements of this essential part of flavors in foods. The chapters fall into the categories of introduction, tea, coffee, citrus and fruit juices and alcoholic beverages and encompass volatile and non-volatiles analyses, sensory and physico-chemistry. Chapter 1 introduction to flavors in noncarbonated beverages includes trends on consumption. Chapter 2 a comparison of teas from Sri Lanka which are especially important to the Japanese market. Chapter 3 non-volatiles in teas. Chapter 4 the newly developed LC-Taste as applied to various topical teas. Chapter 5 non-volatiles in black tea and the healthy properties. Chapter 6 analysis and sensory of a geographically important coffee beans. Chapter 7 Analysis and sensory work on pomegranate juice. Chapter 8 Stability of citral using enhanced cyclodextrin. Chapter 9 Apple juice evaluation. Chapter 10 bioactive components of sea buckthorn. Chapter 11 flavor and processing of blueberries. Chapter 12 odorant release from alcoholic beverages. Chapter 13 detailed analysis of Limoncello liqueur.

We would like to acknowledge the chapter authors who form a nice balance of industry, government and academia which spans four continents; North America, Europe, Asia and Africa. We would also like to acknowledge the following people for their help with the book and symposium, Catherine Hogan, Susan Joseph, Laurence Trinnaman, Mark Dewis, Joseph Leightner, Hernan Vaisman and Cynthia Mussinan of IFF, Jakob Ley of Symrise, Jide Adedeji for co-hosting the original symposium and finally Cheryl Smith for proofing the chapters.

Neil C. Da Costa

Senior Research Fellow
International Flavors & Fragrances Inc.
1515 Highway 36
Union Beach, NJ 07735
neil.dacosta@iff.com (e-mail)

Robert J. Cannon

Research Scientist
International Flavors & Fragrances Inc.
1515 Highway 36
Union Beach, NJ 07735
robert.cannon@iff.com (e-mail)

In Flavors in Noncarbonated Beverages; Da Costa, N., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch001

Chapter 1

Overview of Flavors in Noncarbonated Beverages

Neil C. Da Costa,*[,1] Theodore J. Anastasiou,[2] and Jide Adedeji[3]

[1]International Flavors & Fragrances Inc., 1515 Highway 36, Union Beach, NJ 07748, USA
[2]12 Ladwood Drive, Holmdel, NJ 07733, USA
[3]Transcorp, 11 Alfred Rewane Road, Ikoyi, Lagos, Nigeria
*neil.dacosta@iff.com

Noncarbonated beverages encompass a wide variety of subcategories including teas, iced teas, coffees, fruit juices, water, flavored waters, energy and sports drinks, alcoholic beverages and even dairy drinks. Market share has increased steadily over the years, even to the detriment of carbonated beverages. Within this category the emphasis has shifted towards ready-to-drink (RTD) beverages and what are perceived as healthier drinks such as the waters, energy drinks and drinks high in anti-oxidants such as green teas. This is in line with consumer food and drink trends towards health and wellness. This chapter endeavors to cover the wide variety of beverages in this category, including current trends in their consumption and an emphasis on their key volatile and non-volatile components. A subject as broad as this cannot be done total justice in one chapter and thus the essentials or currently most interesting aspects of many noncarbonated beverages only will be addressed.

Introduction

The beverage market can be split into alcoholic and non-alcoholic segments. The non-alcoholic segment can be further broken down into carbonated beverages, juices, waters, functional drinks, concentrates, dry beverages and ready-to-drink

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch001

(RTD) teas and coffees. Carbonated beverages include soda and seltzers, juices which can be vegetable and fruit based. Waters are bottled with or without flavor, functional drinks are sports and energy drinks, and other beverages designed to offer a health benefit. Concentrates include frozen and powdered drink mixes and RTD packaged teas and coffees.

Global beverage consumption has increased in the period from 2003 to 2008 by 114.3 million liters or 28.1%. The breakdown by beverage category is shown in Table I.

When broken down by region, the beverage market in Asia Pacific and Eastern Europe has recently experienced the largest growth, followed by Latin America and Middle East/Africa, and finally by Western Europe and North America in Table II.

Not all categories have experienced positive growth in all regions. For instance Western Europe saw a decline of 0.4 % for concentrates and 1.8% for RTD tea during the period 2003 to 2008. North America saw declines across more categories during the same period. This is shown in Table III.

North American sales of carbonated beverages are in decline with market growth being driven by bottled water, functional drinks, and RTD teas and coffees. It is forecast that world-wide growth in the carbonated category will be sluggish as consumers shift to other categories due to concern over nutritional issues. Those categories where growth is forecast as a result are bottled water, juices and RTD teas.

Tea

Tea is the highest selling and most consumed noncarbonated beverage globally. It is consumed widely as a warm or hot beverage, but in North America it is popularly consumed cold as the RTD “iced tea”. Amongst RTD teas all regions have shown an increase in consumption except Western Europe, Table IV.

The largest tea producers are China, India, Sri Lanka and Kenya. Most tea leaves are from the genus *Camellia sinensis*, which is sub-divided into *Sinensis sinensis* from China and *Sinensis assamica* from India. There are four classic types of tea, differing in degree of oxidation or fermentation. Black teas (*1*) such as Darjeeling (*2*) and Assam, are the most oxidized, with more flavor and caffeine. They retain their flavor for many years and can be sold at high prices in brick form in China and India, rather like vintage wine. Oolong tea is less oxidized than black tea and is predominantly from China. Green tea is the least oxidized, coming from China and Japan. Its consumption has been increasingly linked to health and wellness. White tea is produced from uncured and unoxidized tea leaf buds and young leaves from China.

In addition there are several herbal teas which technically are not true teas. Herbal teas can be made from fresh or dried flowers, leaves, seeds, or roots of herbs or other plants. Herbal teas as a whole are traditionally considered a panacea, alleviating a plethora of maladies. In addition they are used for their stimulant or sedative properties. Popular herbal teas are those made from mint,

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch001

Table I. Global beverage consumption by category from 2003 to 2008 (Source: © and database right Euromonitor International PLC 2009. All rights reserved.)

Category	*% incr*	*CAGR %*[a]	*Liters (MM)*
Carbonates	12.4	2.4	22,476
Fruit/Vegetable Juice	24.9	4.5	12,152
Bottled Water	45.1	7.7	64,764
Functional Drinks	54.6	9.1	5,000
Concentrates	13.4	2.6	381
RTD Tea	49.8	8.4	8,149
RTD Coffee	21.4	4.0	758
World Total	28.1	5.1	114,314

[a] CAGR is Compounded Annual Growth Rate.

Table II. Global beverage growth by region from 2003 to 2008 (Source: © and database right Euromonitor International PLC 2009. All rights reserved.)

Region	*% incr*	*CAGR %*[a]	*Liters (MM)*
Asia Pacific	49.0	8.3	39,407
Eastern Europe	47.3	8.1	11,792
Latin America	36.7	6.4	26,927
Middle East and Africa	38.0	6.6	10,673
North America	13.1	2.5	13,264
Western Europe	12.6	2.4	11,943

[a] CAGR is Compounded Annual Growth Rate.

rose hip, chamomile, ginseng, chrysanthemum and lemon balm. Other interesting varieties include red tea, or Rooibos tea from South Africa (see Chapter 5.) which lacks caffeine and has a high level of antioxidants. In contrast, yerba maté, produced from a South American shrub, is high in caffeine as well as containing an antioxidant chlorogenic acid mixture (*3*).

The major tea volatiles are linalool, linalool oxides, theaspirane, α-ionone, β-ionone, β-damascenone, geraniol, (*Z*)-3-hexenol, hexanal, (*E*)-2-hexenal, phytol, dihydroactinidiolide, loliolide, indole, hexadecanoic acid, jasmine lactone, and coniferyl alcohol. Non-volatiles present are caffeine, polyphenols (catechins, epicatechins, gallates, amino acids, theanine, saponins), sugars (fructose, glucose, sucrose, maltose), and organic acids (oxalic, citric, malic, succinic, quinic, chlorogenic). Oxidation directly affects the volatile content, with more volatiles being generated with a higher degree of oxidation. (Figures 1 and 2). Note that caffeine is effectively semi-volatile as it can be detected by GC-MS, but is really only quantifiable, like other non-volatiles, by HPLC.

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch001

Table III. North American beverage consumption by category from 2003 to 2008 (Source: © and database right Euromonitor International PLC 2009. All rights reserved.)

Category	*% incr (decr)*	*CAGR*[a]	*Liters (MM)*
Carbonates	(2.9)	(0.6)	(1,689)
Fruit/Vegetable Juice	(11.9)	(2.5)	(1,735)
Bottled Water	57.8	9.6	12,160
Functional Drinks	73.7	11.7	2,889
Concentrates	(10.5)	(2.2)	(5)
RTD Tea	65.5	(10.6)	1,516
RTD Coffee	130.6	18.2	129

[a] CAGR is Compounded Annual Growth Rate.

Table IV. Global RTD Tea consumption by category from 2003 to 2008 (Source: © and database right Euromonitor International PLC 2009. All rights reserved.)

Category	*% incr (decr)*	*CAGRa*	*Liters (MM)*
Asia-Pacific	51.4	8.6	5664
Eastern Europe	355.5	35.4	772
Latin America	125.3	17.6	155
Middle East & Africa	117.3	16.8	66
North America	65.5	10.6	1516
Western Europe	(1.8)	(0.4)	(48)
World Total	49.8	8.4	8149

[a] CAGR is Compounded Annual Growth Rate.

Nantou Oolong (Figure 1), which is the more oxidized tea, has a high level of volatiles. These can be seen in the region between the solvent and caffeine. In contrast, Gyokuro Japanese Green Tea (Figure 2), which is less oxidized, has fewer components at lower concentrations in the same region. As expected in tea, both extracts are dominated by high concentrations of caffeine. Non-volatile analysis of the teas would show a higher content of polyphenols in the green tea as they have not been oxidized to smaller volatile compounds.

The key polyphenols (*4*, *5*) in teas are epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), epigallocatechin-3-gallate (EGCG). Epigallocatechin (see Chapters 3 and 4) can break down to give volatiles like pyrogallol (1, 2, 3-trihydroxybenzene) which can be clearly seen in GC-MS analyses. Depending on the concentration they are found at, these so called polyphenols tend to add bitterness and astringency to all beverages and foods. This is clearly the case with tea.

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch001

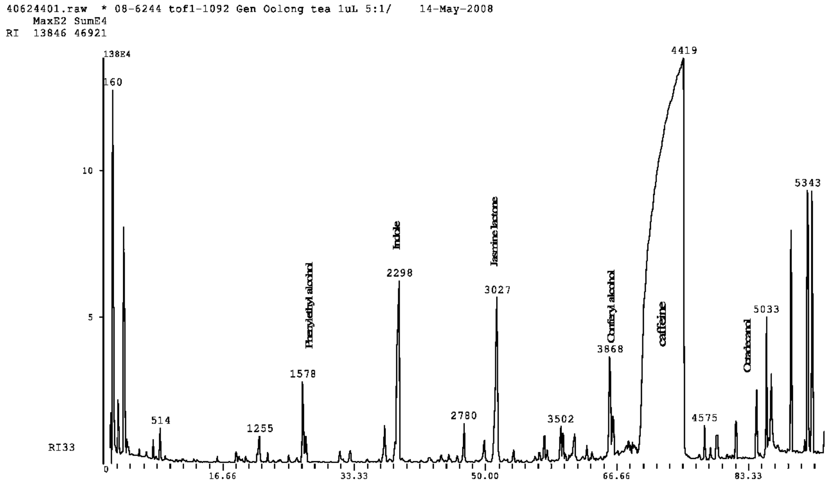


Figure 1. Nantou Oolong tea, liquid/liquid extract chromatogram (Semi-fermented).

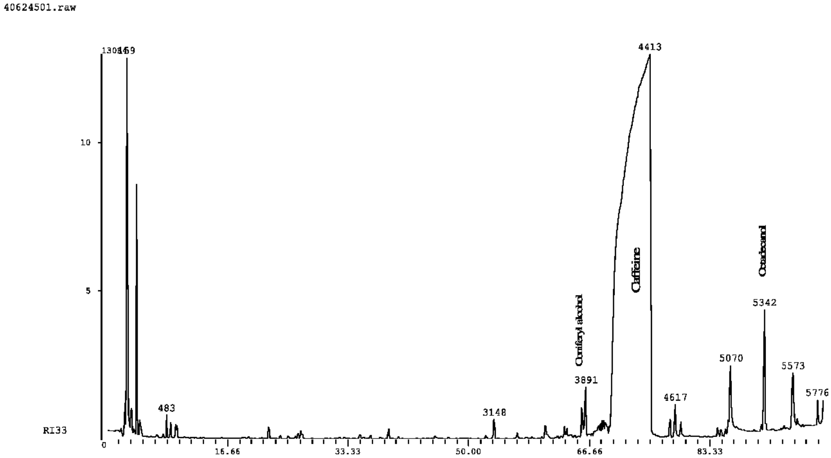


Figure 2. Gyokuro Japanese Green tea, liquid/liquid extract chromatogram (Unfermented).

In addition great interest has been shown in chlorogenic acid (Figure 3) found at particularly high concentration in yerba maté tea (*3*) from South America. Popular particularly in Brazil, Argentina and Paraguay this herbal tea has been reported to have positive health effects due to it high chlorogenic acid concentration, despite the high caffeine content.

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch001

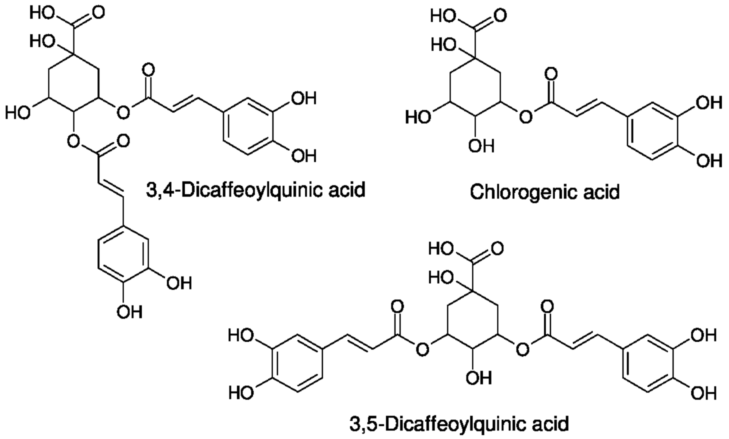


Figure 3. Chlorogenic acid and its analogues.

Coffee

There has been an increase in the popularity of different types of coffee such as instant (freeze-dried), espresso, cappuccino, mocha, iced coffee and flavored coffees, Table V.

The two main types of coffee that are grown are Arabica (*Coffea arabica*) and Robusta (*Coffea canephora*). Arabica is considered to have superior flavor compared to Robusta, but has less body and caffeine. Robusta is often used as a low-cost filler and to provide body to Arabica-based coffees. Arabica coffee is grown throughout Latin America, Central and East Africa, India and Indonesia. Robusta coffee is grown in West and Central Africa, South-East Asia and Brazil. Robusta beans are more disease resistant than Arabica.

After coffee beans are picked they are washed, dried, and then roasted. Roasting affects the taste by causing the loss of some flavor compounds and the formation of others. The beans are produced in several roasts, from light through medium to dark. Darker roasts are generally more bitter, whereas lighter roasts have more caffeine and more aromatic oils and acids which make them more flavorful.

The volatile and non-volatile components of coffee are well documented (*6–9*). The volatile components include furfuryl mercaptan, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, pyridine, pyrrole, thiophenes, β-damascenone, vanillin, methional, guaiacol, 2-acetylfuran, 2-methyl-tetrahydrofuran-3-one, diacetyl, γ-butyrolactone, isovaleraldehyde. The non-volatile components include caffeine, chlorogenic acid, particularly in green beans, trigonelline (see Chapter 6), amino acids, polysaccharides, fructose, glucose, sucrose, fumaric acid, citric acid, phosphoric acid, malic acid, and lactic acid. These components exist in different ratios in Arabica and Robusta coffees, as shown in Figures 4 and 5.

The two chromatograms (Figure 4 and 55) highlight the volatile differences between high quality Kenyan Arabica beans and lower quality Robusta beans. No one single component is responsible for the quality, but differences in the ratios

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch001

Table V. lobal RTD Coffee consumption by category from 2003 to 2008 (Source: © and database right Euromonitor International PLC 2009. All rights reserved.)

Category	*% incr*	*CAGR %*[a]	*Liters (MM)*
Asia-Pacific	15.8	3.0	521
Eastern Europe	162.6	21.3	7
Latin America	102.5	15.2	8
Middle East & Africa	9.3	1.8	0.1
North America	130.6	18.2	129
Western Europe	278.5	30.5	58
World Total	21.4	4	757

[a] CAGR is Compounded Annual Growth Rate.

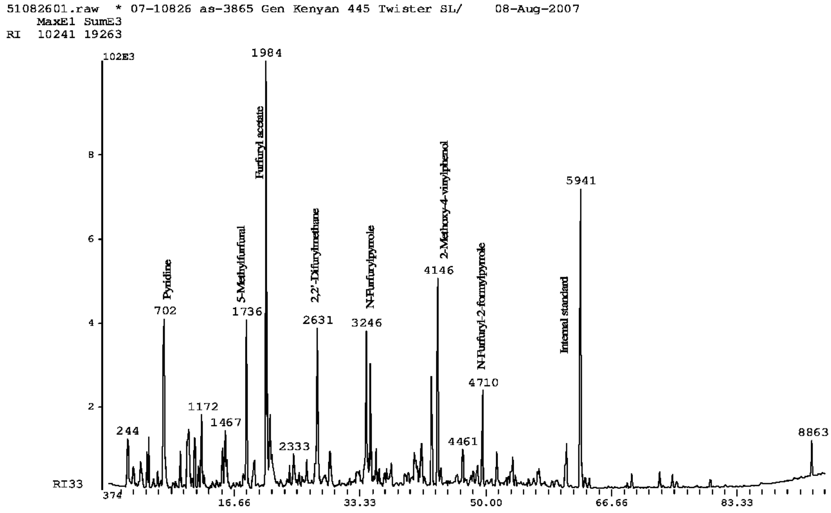


Figure 4. Kenyan Arabica Coffee analyzed by stir-bar sorption extraction.

of various components. Also the non-volatile component trigonelline plays an important role in the nitrogen compounds subsequently generated.

Citrus and Citrus Derived Beverages

Fruit juices contain juices or oils pressed or macerated from the fruit. Fruit juices containing up to 24% juice are the fastest growing sector of juice by volume, while 100% juice is the large value category with growth in China, Brazil, and Mexico.

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch001

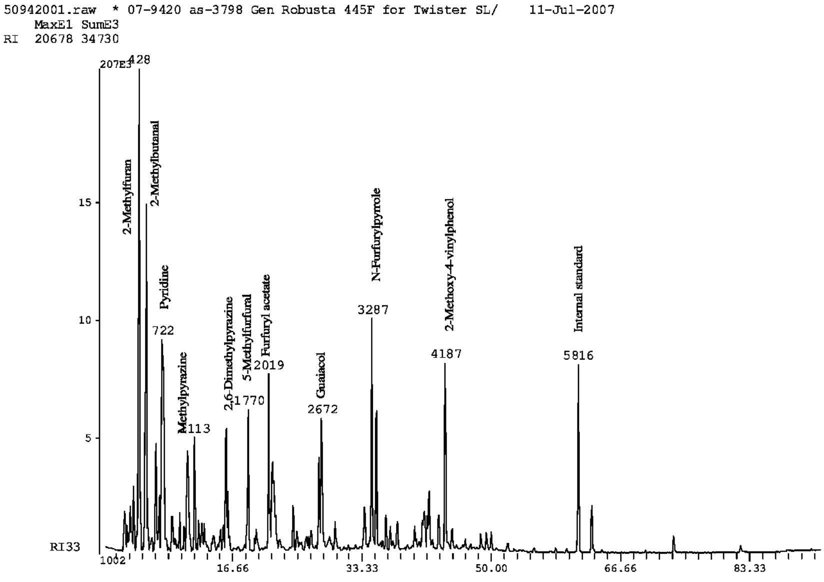


Figure 5. Robusta Coffee analyzed by stir-bar sorption extraction.

Asia Pacific is the fastest rowing region for juices, followed by Eastern Europe, and to a lesser extent Middle East/Africa and Latin America. Growth of juices is sluggish in Western Europe and is negative in North America, Table VI.

Fruit juices are typically canned or pasteurized for shelf life stability (*10*). Since this process requires heat treatment, oils are used more than juices as they are more stable. There are three types of oils derived from citrus fruits: cold press, folded, and washed. Cold press oil is produced from citrus peel by pressing oil out or running fruit over small metal spikes which break the peel's oil sacs and release the oil. Folded oils are less water soluble and more stable. Folded oils are produced by distilling off the front end of the oil under reduced pressure. The more folded the oil the lower the concentration of monoterpenes like limonene and the higher the concentration of sesquiterpenes. Washed oils are produced by partitioning the volatiles between water and ethanol washes. The less ethanol used, the more polar the components found in the aqueous phase of the wash.

Typical volatiles in orange oil (Figure 6) are α-pinene, β-pinene, γ-terpinene, d-limonene, sabinene, β-myrcene, car-3-ene, decanal, octanal, valencene, nootkatone, α-sinensal, β-sinensal. The main fruit varieties are Persian (Mediterranean) orange, Navel orange, Valencia orange, and blood orange. Also south-east Asian (Indonesian) varieties such as Medan and Pontianak (*11*) are gaining popularity.

Lemon oil volatiles (Figure 7) include β-myrcene, α-pinene, β-pinene, γ-terpinene, d-limonene, neral, geranial, nerol, geraniol, β-bisabolene, (E)-α-bergamotene, limettin, bergapten. Protection against degradation of the highly unstable citral (neral/geranial) in lemon applications, is a "holy grail" in flavors (see Chapter 11) Lemon juice itself is a good source of citric acid. Fruit

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch001

Table VI. Fruit juice growth by region from 2003 to 2008 (Source: © and database right Euromonitor International PLC 2009. All rights reserved.)

Region	*% incr (decr)*	*CAGR %*[a]	*Liters (MM)*
Asia Pacific	87.7	13.4	7,905
Eastern Europe	59.3	9.8	2,816
Latin America	35.9	6.3	1,562
Middle East and Africa	40.8	7.1	1,312
North America	(11.9)	(2.5)	(1,735)
Western Europe	2.0	0.4	243
World Total	24.9	4.5	22476

[a] CAGR is Compounded Annual Growth Rate.

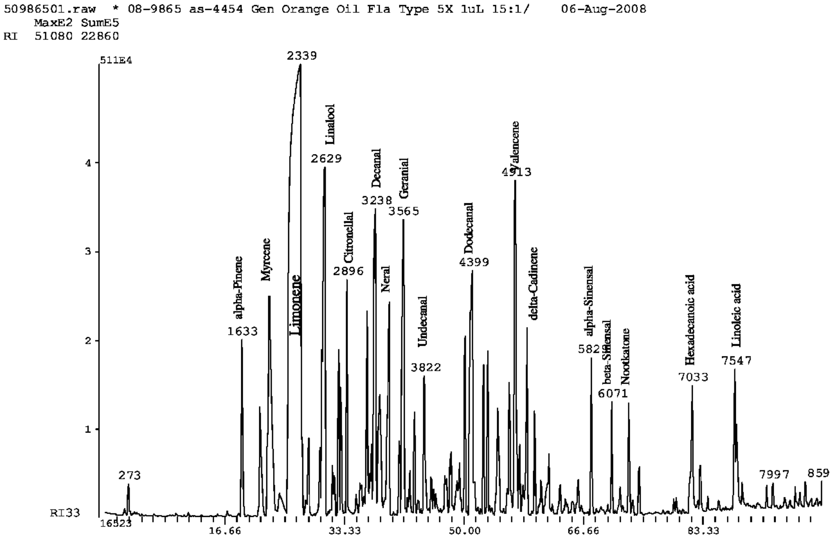


Figure 6. Florida Orange five fold oil chromatogram.

varieties include Meyer lemon and Spanish (Verna) lemon. Lemon oil is used in soft drinks, such as lemonade and alcoholic drinks (see Chapter 13).

Lime oil volatiles (Figure 8) include limetol, d-limonene, α-pinene, β-pinene, β-myrcene, terpinolene, 1,8-cineole, borneol, neral, geranial, neryl acetate, geranyl acetate. Fruit varieties include Mexican, Brazilian, Argentinian, Spanish, Persian and Key lime. Lime oil uses include soft drinks and alcoholic beverages. It is often added to soft lemon drinks.

Grapefruit oil (Figure 9) volatiles are characterized by methyl anthranilate, limonene, nootkatone, grapefruit mercaptan, bergamottin, bergapten and osthole. The main varieties of the fruit are Ruby red, white and pink.

Some of the most interesting citrus non-volatiles are the flavonoid glycosides such as naringin, hesperidin and rutin (Figure 10) found in grapefruit and other

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch001

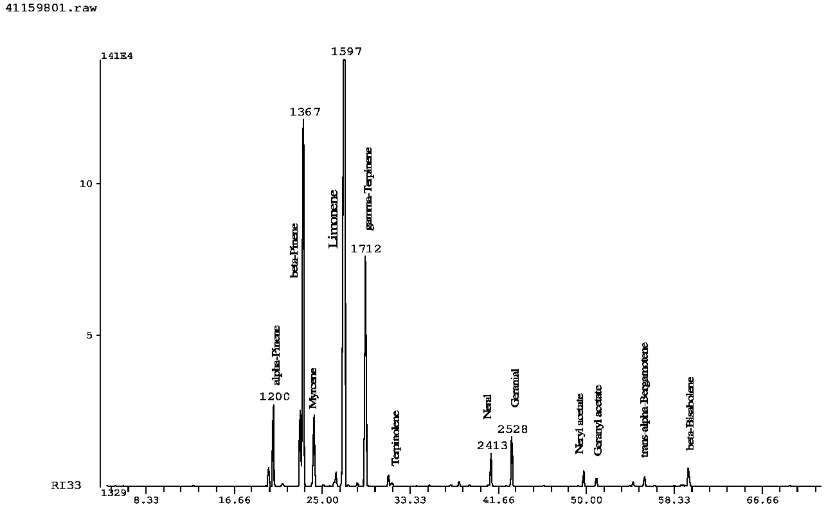


Figure 7. Argentinian Lemon single fold oil (main component is limonene).

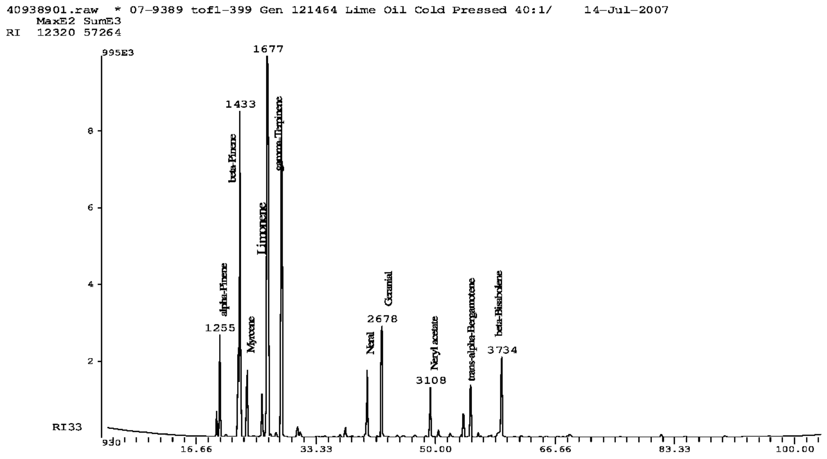


Figure 8. Persian Lime cold press oil.

citrus (*10*). They are reported to have health benefits such as anti-inflammatory, anti-cancer, to lower blood pressure and lower cholesterol (*12*).

Other Fruit Based Beverages

The most popular berry flavors for beverages are strawberry (Figure 11), blackberry, raspberry, blueberry (see Chapter 10) and cranberry. Their most characteristic volatiles are α-ionone, β-ionone, β-damascenone, damascones, irones, furaneol™, (*Z*)-3-hexenol, ethyl butyrate and maltol. Non-volatiles

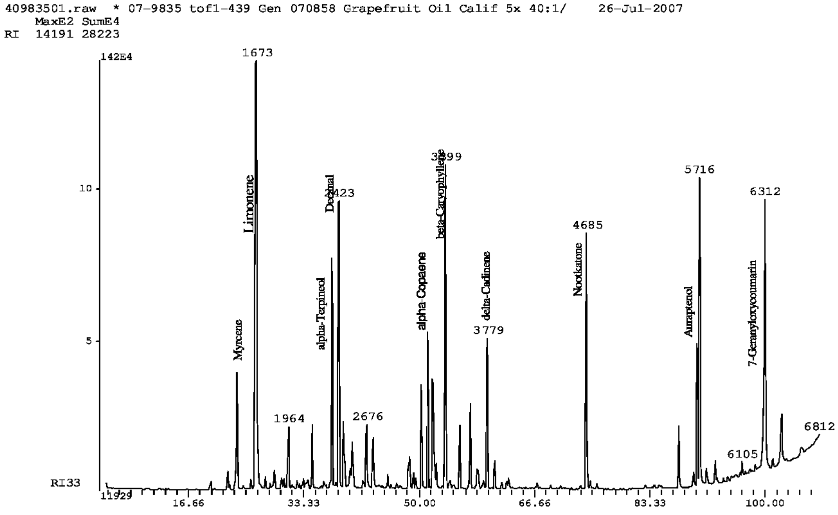


Figure 9. Californian Grapefruit five fold oil.

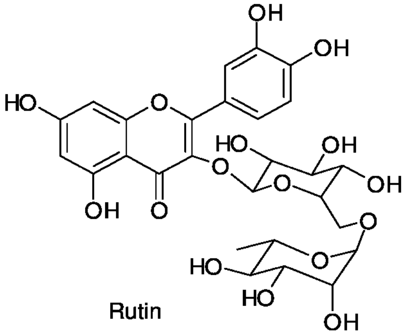


Figure 10. Flavonoid glycoside, Rutin.

include glucose, fructose, sucrose, polyphenols, ascorbic acid, citric acid and anthocyanins, which give color (*13*).

Grape flavors are characterized by anthranilate esters such as methyl anthranilate, and methyl N-methylanthranilate. Also trace sulfur molecules like 4-methyl-4-mercaptopentan-2-one (3) in Figure 12. reported in Sauvignon grapes. Cherry flavors usually contain high concentrations of benzaldehyde and anisaldehyde. Apple juice (see Chapter 8) is another cheap to produce, popularly consumed beverage (*14*) and pear juice is commonly used as an extender in many other fruit juices.

The following fruits have been found to contain trace levels of specific sulfur compounds. In some cases they are specific to that fruit. Peach, apricot, mango (*15*) (ethyl 3-mercaptobutyrate, 2), blackcurrant (4-methoxy-2-methylbutan-2-thiol, 4), guava and passionfruit (3-(methylthio)-hexyl butyrate, 1 and 2-methyl-4-propyl-1,3-oxathiane, 8) are characterized by these trace sulfur molecules (Figure 12), which it is difficult to create good flavors without (*16*).

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch001

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch001

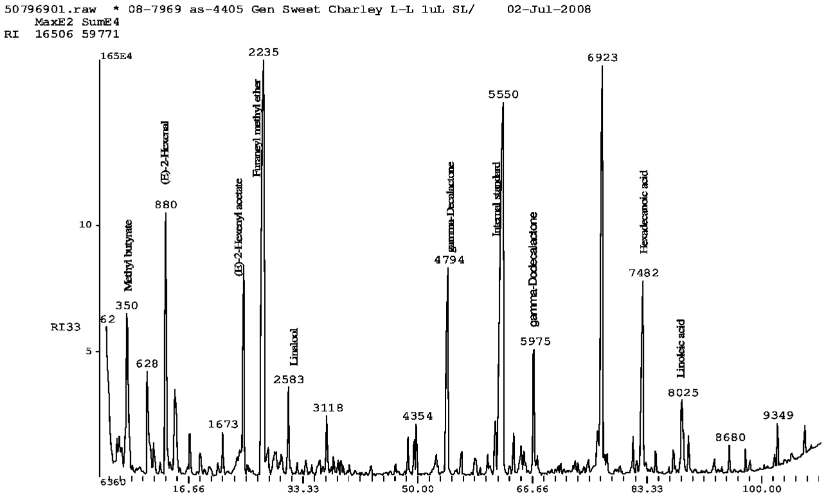


Figure 11. Strawberry Sweet Charley Liquid/liquid Extract.

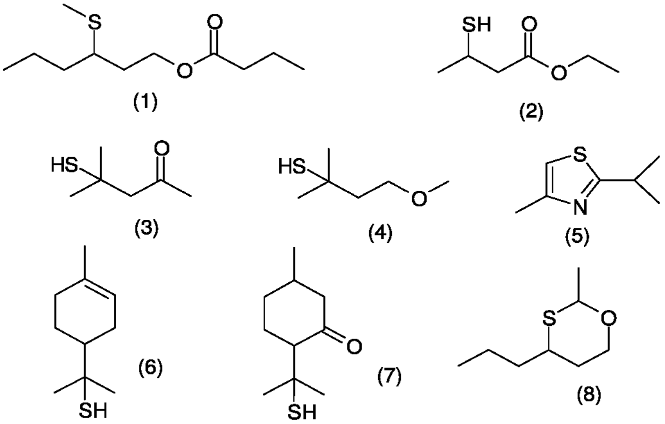


Figure 12. Key sulfur volatiles in fruits.

Other sulfur compounds that can be added to give effect are grapefruit mercaptan (6), 2-isopropyl-4-methylthiazole (5) and buchu oil which contains the active, p-menthan-8-thiol-3-one (7).

Pineapple juice contains bromelain a sulfur containing protein digesting enzyme. It can break down protein to give smaller non-volatile compounds (*17*) such as S-sinapyl glutathione (Figure 13) and thus onto volatiles. Bromelain also has reported health benefits (*18*).

Superfruits are considered to be fruits possessing potential health benefits and usually display antioxidant properties. Such fruits include the more familiar cranberry, blueberry, elderberry, kiwi and pomegranate (see Chapter 7). Less commonly included are açai, a South American berry made into a juice or pulp; guarana a South American fruit used to make a herbal tea, high in caffeine; wolfberry or goji, a Chinese fruit made into tea or even wine; noni, a Pacific island fruit made into a juice or wine; and sea-buckthorn, an Asian fruit consumed as a juice (see Chapter 9). The antioxidant properties of their polyphenols are thought to act as free radical scavengers and thus contribute to health and wellness.

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch001

Miscellaneous

Sports & Energy Drinks

An increasingly popular part of the beverage market are so called sports and energy drinks. They are supposed to display positive effects when consumed, such as increased energy, improved mental and cognitive state, alertness and even euphoria. However they have also been known to display negative effects such as nervousness, irritability, difficulty sleeping, arrhythmia, upset stomach and even seizures, particularly when consumed in excess. Their consumption has greatly increased across all regions from 2003 to the present, Table VII.

Popular energy and sports drinks include older brands such as Lucozade®, Irn-Bru®, Gatorade® and more modern ones such as Red bull®. Variants of the latter tend to contain higher concentrations of stimulants such as caffeine, herbal supplements, guarana, ginseng, glucuronolactone, carnitine, creatine, vitamins, plus sugars and other sweeteners (Figure 14).

Flavored Water

As shown in Table I. bottled waters have shown a 45% increase from 2003 to the 2008 and are one of the highest increases in beverage consumption as they are perceived to be healthier than tap water. Consequently flavored water consumption has increased for those who do not like the taste of plain water. The most popular flavors are fruits and berries, sometimes fortified with vitamins, other supplements and low calorie sweeteners.

Dairy Beverages

Popular diary beverages include the well established milkshakes and more recently yogurt drinks. They are often flavored with fruits, berries, vanilla and chocolate. Incorporating lactobacillus cultures has lead to reported health digestive benefits (*19*).

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch001

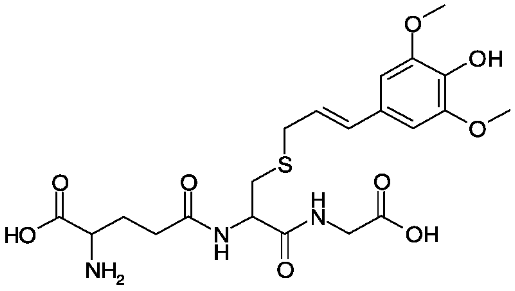


Figure 13. S-sinapyl glutathione peptide from pineapple.

Table VII. Global Sports and Energy drinks consumption by category from 2003 to 2008 (Source: © and database right Euromonitor International PLC 2009. All rights reserved.)

Category	*% incr*	*CAGR %*[a]	*Liters (MM)*
Asia-Pacific	36.1	6.7	531
Eastern Europe	390.4	46.5	153
Latin America	343.4	41.8	452
Middle East & Africa	237.1	43.8	172
North America	409.8	44.4	2876
Western Europe	155.9	24.0	710
World Total	169.4	25.1	4971

[a] CAGR is Compounded Annual Growth Rate.

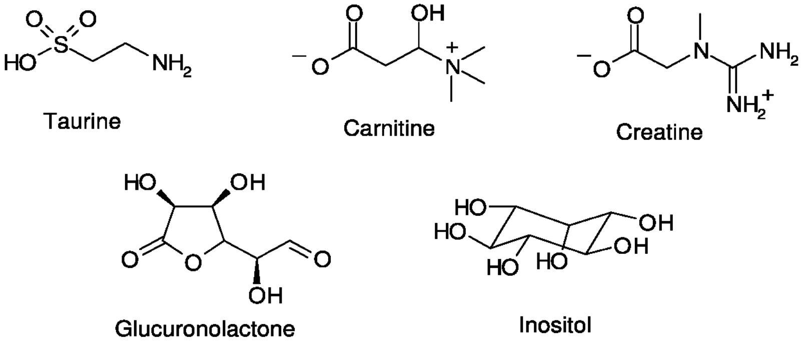


Figure 14. Key Non-volatile constituents of Energy Drinks.

Soy milk and related beverages are popular alternatives to cow's milk for vegans. They contain no cholesterol and are more suitable for lactose intolerant consumers. Without flavor they tend to be less palatable to most consumers, often described as green, beany, bitter and astringent. When flavoring, dosages need to be high as the flavor tends to bind to soy proteins leading to flavor loss over time.

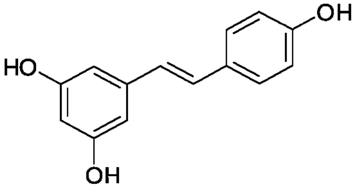


Figure 15. Resveratrol structure.

Alcoholic Beverages

This group of beverages fits into noncarbonated beverages, but would deservedly earn the right to its own book to cover the wide variety of drinks available and thus be beyond the scope of this brief chapter. Red wine is the most commonly reported alcoholic beverage displaying beneficial health effects. It is high in polyphenols (*20*), the most topical being resveratrol (Figure 15) reputedly possessing anti-cancer, anti-inflammatory, blood sugar lowering properties (*21*).

Summary

This chapter has endeavored to cover the main sub-categories of noncarbonated beverages. It is a very wide subject for which specific flavor topics were referenced as they are covered in greater detail in some of the following chapters. New and exciting molecules are still to be discovered, with important flavor properties and greater health claims still to be made. The move to more exotic and healthily perceived beverages seems to be a trend here to stay for the foreseeable future.

References

1. Owour, P. O. Flavour of black tea – A review. *Tea* **1986**, *7* (1), 29–42.
2. Schuh, C.; Schieberle, P. Characterization of the key aroma compounds in the beverage orepared from Darjeeling black tea: Quantitative differences between tea leaves and infusion. *J. Agric. Food Chem.* **2006**, *54*, 916–924.
3. Heck, C. I.; de Mejia, E. G. Yerba Maté tea (*Ilex paraguariensis*): A comprehensive review on chemistry, health implications, and technological considerations. *J. Food Sci.* **2007**, *72* (9), 138–151.
4. Fernandez, P. L.; Martin, M. J.; Gonzalez, A. G.; Pablos, F. HPLC determination of catechins and caffeine in tea. Differentiation of green, black, and instant teas. *Analyst* **2000**, *125*, 421–425.
5. Lakenbrink, C.; Lapczynski, S.; Maiwald, B.; Engelhardt, U. H. Flavonoids and other polyphenols in consumer brews of tea and other caffeinated beverages. *J. Agric. Food. Chem.* **2000**, *48*, 2848–2852.

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch001

6. Flament, I. Coffee, Cocoa, and Tea Flavors: A Review of Present Knowledge. In *Coffee Flavor Chemistry*; John Wiley & Sons, Ltd.: Chichester, England, 2002; p 424.
7. Clarke, R. J., Vitzthum, O. G., Eds.; *Coffee Recent Developments*, World Agriculture Series; Blackwell Science, Ltd.: Oxford, England, 2001; p 272.
8. Sturdivant, S. 10th annual coffee and tea flavor review. *Tea Coffee Trade J.* **2001**Jan/Feb, *173* (1), 63–72.
9. Kerler, J. Review of coffee aroma chemistry. *Colloq. Sci. Int. Café* **2006**, *21*, 328–331.
10. Ashurst, P. R., Ed.; *Chemistry and Technology of Soft Drinks and Fruits Juices*, 2nd ed.; Blackwell Publishing, Ltd.: Oxford, England, 2005.
11. Dharmawan, J.; Kasapis, S.; Sriramula, P.; Lear, M.J.; Curran, P. Evaluation of aroma-active compounds in Pontianak orange peel oil (*Citrus nobilis* Lour. var. *microcarpa* Hassk.) by gas chromatography−olfactometry, aroma reconstitution, and omission test. *J. Agric. Food Chem.* **2009**, *57* (1), 239–244.
12. Tomasik, P., Ed.; *Chemical and Functional Properties of Food Saccharides*; CRC Press: Boca Raton, FL, 2004; p 389.
13. Buchert, J.; Koponen, J. M.; Suutarinen, M.; Mustranta, A.; Lille, M.; Törrönen, T.; Poutanen, K. Effect of enzyme-aided pressing on anthocyanin yield and profiles in bilberry and blackcurrant juices. *J. Sci. Food Agric.* **2005**, *85*, 2548–2556.
14. Heil, M.; Ara, V. Aroma of fruit juices II – Composition and valuation of apple juice aroma. *Fruit Process.* **2008**, *18* (3), 126–134.
15. Dewis, M. L.; Kendrick, L. Creation of Flavours and the Synthesis of Raw Materials Inspired by Nature. In *Advances in Flavours and Fragrances – From Sensation to the Synthesis*; Swift, K. A. D., Ed.; The Royal Society of Chemistry: U.K., 2002; pp 147−160.
16. Jameson S. B. Aroma chemicals III: Sulfur compounds. In *Chemistry and Technology of Flavors and Fragrance*; Rowe, D.J., Ed.; Blackwell: Oxford, U.K., 2003; pp116−142.
17. Wen, L.; Wrolstad, R.E.; Hsu, V. L. Characterization of sinapyl derivatives in pineapple (*Ananas comosus* [L.] Merill) juice. *J. Agric. Food Chem.* **1999**, *47* (3), 850–853.
18. Kleef, R.; Delohery, T.; Bovbjerg, D. Selective modulation of cell adhesion molecules on lymphocytes by bromelain protease 5. *Pathobiolog.* **1996**, *64* (6), 339–346.
19. Ljungh, A., Wadstrom, T., Eds.; *Lactobacillus Molecular Biology: From Genomics to Probiotics*; Caister Academic: U.K., 2009; p 205.
20. Robicaud, J. L.; Noble, A. C. Astringency and bitterness in selected phenolics in wine. *J. Sci. Food Agric.* **1990**, *53*, 343–353.
21. Baur, J .A.; Sinclair, D. A. Therapeutic potential of resveratrol: The in vivo evidence. *Nat. Rev. Drug Discov.* **2006**, *5* (6), 493–506.

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch001

Downloaded by CORNELL UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch002

Chapter 2

Characteristic Odorants of Sri Lankan Black Teas

Uva, Nuwara Eliya, Dimbula, Kandy, and RuhunaVarietals

Hisae Kasuga,* Yuichiro Yamazaki, Aki Baba, and Sachiko Okajima

Takasago International Corporation, Corporate Research & Development Division, 4-11, 1-Chome, Nishi-Yawata, Hiratsuka City, Kanagawa 254-0073, Japan
***hisae_kasuga@takasago.com**

The characteristics and flavor profiles of five types of Sri Lankan teas were investigated by means of GC/FID, GC/MS, GC/O, MDGC and GCxGC. The key compounds identified were linalool, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (4HDMF, furaneol), geraniol, vanillin, (*E*)-2-hexenoic acid, 3-methylbutyric acid (isovaleric acid) and phenylacetic acid. The key specific compounds of high-grown teas were rich with floral, fresh and sweet compounds such as *β*-damascenone, jasmonates, methyl salicylate, lactones and so on. Medium and lower elevation grown teas contained more nutty and smoky note compounds. (*R*)- and (*S*)-linalool were in a ratio of about 60:40, while *γ*-lactones were determined as almost racemates in all the teas. 4HDMF was wide-ranging in concentration from 11-1,200µg, and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (3HDMF, sotolon) was determined at sub µg order in 1L infusions.

Introduction

The production of *Camellia sinensis* tea from Sri Lanka is only second to India when it comes to the amount of tea exported. Sri Lanka is one of the most important black tea producers globally; especially in Japan where 60% of all black

In Flavors in Noncarbonated Beverages; Da Costa, N., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

tea is imported from Sri Lanka. The Japanese tend to prefer the superior aroma and taste of Sri Lankan black tea.

Thus Sri Lankan black tea is very popular. People often think of Sri Lankan black tea as only one type of tea flavor, but there are five major types of teas in Sri Lanka and they each have their own distinctive flavor. The impact of the aroma and taste that each tea gives is quite different between each type.

The black tea grade of Sri Lanka is classified into three types: high-grown, medium-grown, and low-grown, depending on the sea level where the tea plantation is located. In general, it is said that the tea grown on the higher ground has a better quality (*1*).

- High-grown teas: produced 1,200m above sea level, are the best that Sri Lanka produces, giving a beautiful color and an intensely powerful flavor. (Uva, Nuwara Eliya, Dimbula varietals).
- Medium-grown teas: produced between 600m and 1,200m, are rich in flavor and give a good color. (Kandy varietal).
- Low-grown teas: produced only 600m above sea level, are still of good quality and black color, and usually used for blending. (Ruhuna varietal).

Although the flavor and the taste of Uva tea, which is the best quality of Sri Lanka, has been reported many times previously (*2*–*6*), only a few studies have been reported on the flavor of Dimbula tea (*7*–*9*) amongst other black teas of Sri Lanka. Particularly reported were flavor studies using the brewed extract method (*9*), which duplicates the original tea flavor, have rarely been reported except for Uva tea (*10*). For the further development of the black tea flavor of each province, we studied the characteristics and the profiles of the flavor of Uva, Nuwara Eliya, Dimbula, Kandy and Ruhuna.

Downloaded by CORNELL UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch002

Experimental

Materials

The tea samples were purchased from local tea importers in Japan. Uva was produced at the St. James tea garden in Uva province. Dimbula was produced at the Petiagara tea garden in Dimbula province and Nuwara Eliya was produced in Nuwara Eliya province; all in 2008. Kandy tea was produced at the tea garden in Kandy province and similarly Ruhuna was produced at the tea garden in Ruhuna province; both in February 2008. Nuwara Eliya, Uva and Dimbula are also described as Orange Pekoe teas. Kandy and Ruhuna are Broken Orange Pekoe teas. The important compounds for GC/MS identification were obtained from commercial sources. [^{2}H]-3HDMF was synthesized (*11*) in our laboratory.

Sensory Test

200mL of the hot water (85-90°C) was poured onto 5g of the tea leaves in a 450mL volume glass pot. After 5 minutes, the leaves were separated using a

Table 1. Calibration Curves of 4HDMF in Each Tea

Sample	*Approximate equation*	R^2
Nuwara Eliya	$y = 0.0949x + 0.6943$	0.977
Uva	$y = 0.1049x + 1.8409$	0.974
Dimbula	$y = 0.0901x + 9.3052$	0.954
Kandy	$y = 0.0822x + 0.9313$	0.987
Ruhuna	$y = 0.0400x + 3.1698$	0.756

tea strainer and the infusion was divided into seven panellers' cups. Seven expert flavorists at our company smelled and tasted the infusions. They evaluated the odor, the flavor and the color using standard descriptors.

Sample Preparation (Brewed Extract Method)

75mL of hot water (85-90°C) was added to 5g of tea leaves. After 10 minutes, the leaves were separated using a tea strainer. The infusion was immediately cooled to room temperature in ice water. The infusion was topped up to 75mL, and 50μg of nonan-3-one was added as an internal standard (ISTD). The solution was saturated with sodium chloride and was extracted four times with 5mL of dichloromethane solvent. The combined extracts were dried over with anhydrous sodium sulfate, filtered, and the solvent carefully reduced by evaporation to 5mL. This concentrate was extracted with diethyl ether and the ether phase was cooled in a refrigerator to remove precipitating caffeine. It was then concentrated using a stream of nitrogen after Kuderna-Danish concentration to a final volume of 100μL. The aroma extracts were subjected to various analytical instrumental techniques.

Instrumental Analysis

Gas Chromatography–Mass Spectrometry (GC/MS); a GC/MS-QP2010 gas chromatograph and mass spectrometer (Shimadzu Corp.) was employed with helium carrier gas at 110kPa pressure. Samples were injected 20:1 split onto a 50m × 0.25mm I.D.× 0.15μm df , BC-WAX capillary column (GL Sciences Inc.). The oven temperature started at 70°C, and increased to 215°C at a rate of 4 °C/min. Compounds were identified by mass spectral match and the comparison of retention times with authentic samples.

Gas Chromatography (GC); a HP6890 gas chromatograph (Agilent Technologies) equipped with flame-ionization detector (FID) was used with helium carrier gas at 75kPa pressure. Samples injected 20:1 split onto a 25m × 0.2mm I.D. × 0.1μm df , HP-20M capillary column (Agilent Technologies). The oven temperature started at 70 °C, and increased to 215 °C at a rate of 4°C/min. The GC/FID peaks were identified using the GC/MS results and the retention time compared to those of authentic samples.

Downloaded by CORNELL UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch002

Downloaded by CORNELL UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch002

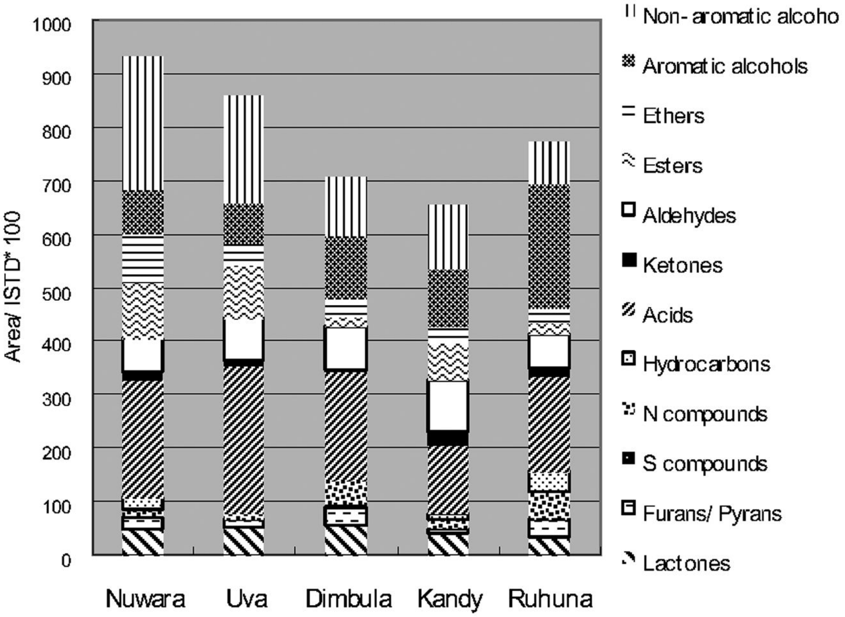


Figure 1. A stacked bar graph of functional groups in five types of tea.

Gas Chromatography-Olfactometry (GC/O); a GC353B (GL Sciences Inc.) equipped with an FID and a sniffing port was used, again with helium carrier gas at 40kPa pressure. Samples were injected 10:1 split onto a 30m × 0.53mm I.D. × 1.0µm df, BC-WAX capillary column (GL Sciences Inc.), then split 1:10 (detector: sniffing port). The oven temperature started at 35°C, and increased to 230°C at a rate of 4°C/min. To determine the potent odorants of teas, AEDA (Aroma Extract Dilution Analysis) was performed in the way described below.

Comprehensive two-dimensional gas chromatography-Mass spectrometry (GCxGC/MS); a 7890A GC system and 5975C inert XL EI/CI MSD (Agilent Technologies) was employed with helium carrier gas at 685kPa pressure. Samples were injected 20:1 split onto the first column, 50m × 0.25mm I.D. × 0.15µm df, BC-WAX (GL Sciences Inc.), then separated each 5sec by a modulator and transferred to the second column, 2.0m × 0.1mm I.D. × 0.10 µm df InertCap 5 (GL Sciences Inc.). The oven temperature started at 80°C, and was increased to 225°C at a rate of 4°C/min. Some low concentration, key compounds were identified by GCxGC/MS.

AEDA (Aroma Extract Dilution Analysis)

The volatiles extracted from a tea were diluted stepwise 4-fold with diethyl ether by volume then subjected to a GC/O analysis. The flavor dilution (FD) factor of each odorant was determined. To identify the compounds, the odor description during GC/O analysis was confirmed by comparing to injected authentic sample aromas, as well as the retention time and mass spectra.

Chiral Analysis

Linalool and various lactones were injected into a Multi Dimensional Gas Chromatography-Mass spectrometry (MDGC/MS) to determine the ratio of (*R*)-isomer:(*S*)-isomer. Chiral analyses were performed using a MDGC-2010 (Shimadzu corp.) equipped with an FID for the first GC and mass spectrometer for the second GC with helium carrier gas at 180kPa pressure. Samples were injected 5:1 split injection onto a 30m × 0.25mm I.D. × 0.25μm df , BC-WAX capillary column (GL Sciences Inc.). The target compounds were transferred to 25m × 0.25mm I.D. × 0.25μm df, CP-Chirasil-Dex CB (Varian) with 130kPa switching pressure. The first oven temperature started at 70°C, increased to 230°C at a rate of 5°C/min. The second oven temperature started 70°C, increased to 150°C at a rate of 1°C/min. The enantiomers were separated base-to-base and the ratios were calculated by the peak areas of the total ion chromatogram.

Quantitative Analysis of 4HDMF and 3HDMF

The volatile aroma extracts of the teas were prepared using the procedure described in sample preparation section. However 20g of tea leaves were used in 300mL hot water, and 0.5μg ethyl maltol and 0.8μg [^{2}H]-3HDMF were added as internal standards. 4HDMF was quantified by the method of standard addition and 3HDMF was quantified by Stable Isotope Dilution Assay (SIDA) (*12*) using GCxGC/MS-SIM (Selected Ion Monitoring). The parameters of the GCxGC/MS were the same as those described in the instrumental analytical section except using SIM mode and detecting specific ions for each compound (ethyl maltol; *m/z* 125, 139, 140, 4HDMF; 57, 85, 128, 3HDMF; 55, 83, 128, [^{2}H]-3HDMF; 59, 87, 132). The response ratio of 3HDMF/[^{2}H]-3HDMF was 0.32 with a standard deviation of ±0.02 over 5 repeated experiments. The calibration curves of 4HDMF were determined for each tea extract (Table 1). The quantitative analysis of each tea extract was repeated 3 times.

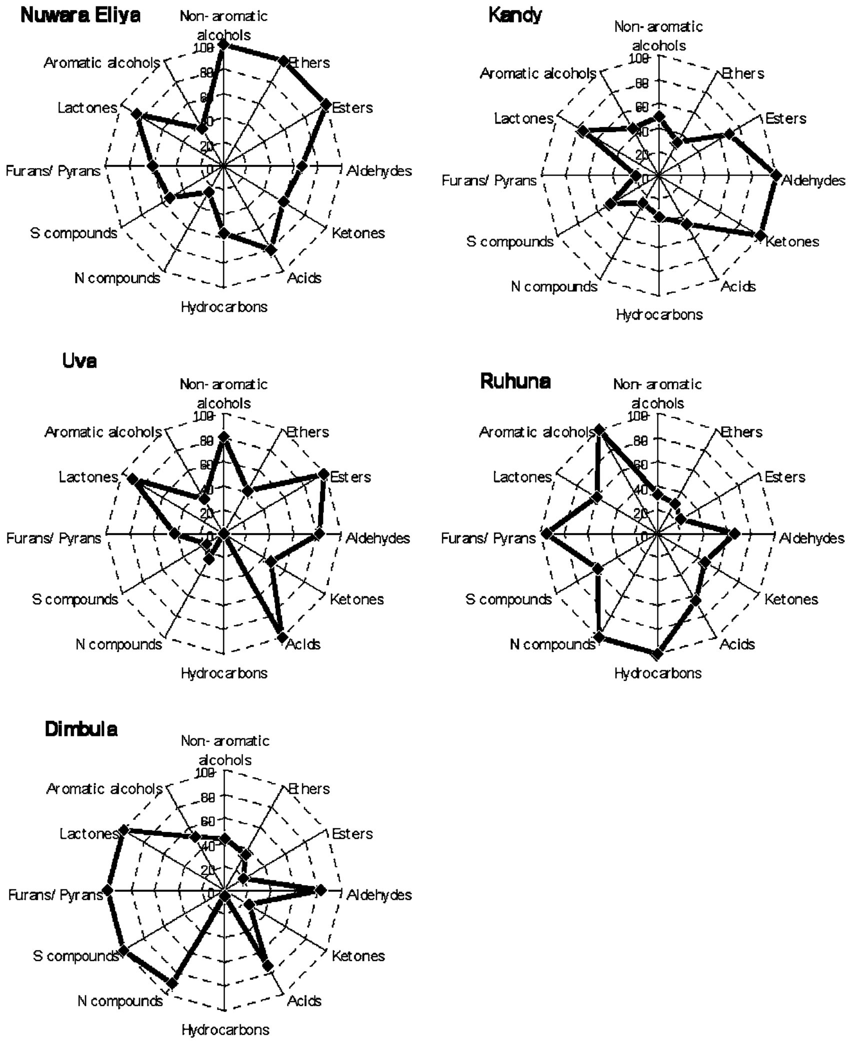
Results and Discussion

The sensory characteristics of the five teas were as follows.

- Nuwara Eliya; Best quality, pale color, rich aroma, fresh green, floral, fruity notes.
- Uva; Sharp minty aroma (so-called Uva flavor) with fruity peach and rose notes.
- Dimbula; Sweet, rich, rosy notes reminiscent of baked sweet potato.
- Kandy; Rich color, less odor, fresh cucumber notes.

Downloaded by CORNELL UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch002

Downloaded by CORNELL UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch002



Nuwara Eliya

Kandy

Uva

Ruhuna

Dimbula

Figure 2. Spider charts of the functional groups of five teas.

- Ruhuna; Smoky, phenolic, scent of withered leaves, soy sauce notes.

A total of two hundred and fifty compounds were identified in the extracts of the five teas by GC/MS and GCxGC/MS analysis.

The peak areas of the detected compounds in the five teas were normalized using the ISTD, and then sorted into functional groups. A stacked bar graph of functional groups in the five types of tea is shown in Figure 1. Spider charts of the functional groups of five teas are shown in Figure 2. In the spider chart, the total area of a functional group in each tea was compared with that of the others and the maximum total area of the functional group was standardized to one hundred.

Nuwara Eliya and Uva had a higher concentration of fresh, green, floral terpene and aliphatic alcohols such as linalool, (*E*)-2-hexenol and

Downloaded by CORNELL UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch002

Table II. FD Factors of Aroma Compounds in Tea Aroma Extracts

		FD factor (>16)				
*L.R.I.**	*Compound*	*Nuwara Eliya*	*Uva*	*Dim-bula*	*Kandy*	*Ruhuna*
1122	(*Z*)-3-Hexenal**	64	64	16	64	64
1328	2,3-Dimethylpyrazine			64		64
1359	4-Mercapto-4-methylpentan-2-one**		16	16	16	64
1363	(*Z*)-3-Hexenol	64	64	16	16	16
1424	2-Ethyl-3,6-dimethylpyrazine	16	16	64	16	
1428	Methional	16		256	64	256
1446	2-Ethyl-3,5-dimethylpyrazine			64		16
1470	(*E*,*E*)-2,4-Heptadienal	64	64	64	64	64
1496	Unknown (green, metallic)		64	16	16	16
1560	(*E*,*Z*)-2,6-Nonadienal	16	64		64	
1562	Linalool	1024	1024	256	256	64
1596	Acetylpyrazine					64
1614	Phenylacetaldehyde	16	16	64	64	16
1643	isoValeric acid	256	64	64	64	256
1676	3-Methylnonan-2,4-dione	16	64	64	64	16
1679	(*E*,*E*)-2,4-Nonadienal				64	
1741	Methyl salicylate	16	64			
1802	*β*-Damascenone	64	64	1024	16	64
1815	3-Mercaptohexanol**	64	16	16	16	16
1820	Hexanoic acid	16	64	64	16	64
1827	Geraniol	64	256	64	64	16
1830	Guaiacol	64				64
1885	2-Phenylethyl alcohol	64	16	16	64	64
1910	(*Z*)-3-Hexenoic acid	64	64	64	64	16
1917	*β*-Ionone	64	64	16	64	
1933	(*E*)-2-Hexenoic acid	64	64	64	64	256
1960	4,5-Epoxy-2-decenal (isomer 1)	64	64		16	64
1973	4,5-Epoxy-2-decenal (isomer 2)	64	16	16	64	16
1992	*γ*-Nonalactone	256	16		64	
1996	4HDMF (Furaneol)	256	64	1024	256	1024
2113	*γ*-Decalactone	64			16	
2142	Eugenol	64		16	16	64
2156	3HDMF (Sotolon)	256	64	16		
2162	4-Vinylguaiacol	64	16		16	64
2306	Methyl jasmonate	64				
2313	(*E*)-Isoeugenol	64				
2364	Methyl epi-jasmonate	64	16			
2511	Phenylacetic acid	256	16	64	64	16
2918	Vanillin	256	64	64	256	64
2819	Raspberry ketone	16			256	

* L.R.I.(Linear Retention Indices); relative to the series of n-hydrocarbons on 50m × 0.25mm I.D. × 0.15μm df , BC-WAX capillary column (GL Sciences Inc.). ** Tentative identification by the odor description & L.R.I. of authentic samples by means of GC/O.

(*Z*)-3-hexenol than the others, while Ruhuna had more aromatic alcohols such as 2-phenylethanol, benzyl alcohol, phenol, guaiacol and dihydroconiferyl alcohol.

Table III. Ratios of Enantiomers of Chiral Compounds*

	The ratio of (R) : (S)				
Compound	*Nuwara Eliya*	*Uva*	*Dimbula*	*Kandy*	*Ruhuna*
Linalool	60 : 40	58 : 42	64 : 36	58 : 42	60 : 40
γ-Nonalactone	46 : 54	47 : 53	49 : 51	46 : 54	49 : 51
γ-Decalactone	52 : 48	48 : 52	54 : 46	55 : 45	58 : 42

* Standard deviations were ±0.1-3.9 with each measurement repeated three times.

Downloaded by CORNELL UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch002

Compounds with ether functions largely comprised the linalool oxides. Nuwara Eliya that had highest concentration of linalool, and correspondingly its oxides.

With regard to ester content, the highest concentration of methyl salicylate was detected in Uva followed by Nuwara Eliya. Nuwara Eliya had a greater concentration of methyl jasmonate with a green floral note than the others. Dimbula and Ruhuna possessed lower concentrations of esters.

With regard to aldehydes and ketones, Kandy had a lot of them especially hexanal and (*E*)-2-hexenal with green notes. It seemed that the characteristics of the Kandy aroma were composed mainly of aldehydes, ketones, less alcohols and less sweet compounds like 4HDMF.

Nuwara Eliya contained more fatty acids than the others after Uva. Ruhuna possessed the highest concentration of aliphatic hydrocarbons.

Dimbula contained many furans, pyrans, nitrogen compounds (pyrazines and pyrroles), sulfur compounds (methional), lactones and vanillin. Combined, they were thought to contribute to the characteristic roasted sweet potato aroma of Dimbula.

Similarly to Dimbula, Ruhuna contained a large concentration of furans, pyrans, and nitrogen compounds. But it had less methional and more aromatic alcohols. It seemed to show only roasted and phenolic note without the sweet potato scent.

The FD factors of aroma compounds in tea aroma extracts over sixteen are shown in Table II.

According to the AEDA results, high aroma contributing compounds for black tea were found to be as follows: (*Z*)-3-hexenal, (*Z*)-3-hexenol with leaf-green notes, linalool and geraniol with floral-green notes, phenylacetaldehyde, 2-phenylethyl alcohol with floral-rose notes, *β*-damascenone, phenylacetic acid with floral-honey notes, 4HDMF, 3HDMF, vanillin with sweet notes, 4-vinylguaiacol, guaiacol, eugenol with phenolic notes, isovaleric acid, hexanoic acid, (*E*)-2-hexenoic acid, (*Z*)-3-hexenoic acid with sweaty acid notes and (*E*,*E*)-2,4-heptadienal, 3-methylnonane-2,4-dione, 3-mercaptohexanol, 4,5-epoxy-2-decenal, (*Z*,*E*)-3,5-octadien-2-one with hay-oily notes.

The characteristics of each tea aroma on AEDA results were as follows.

- Nuwara Eliya; lactones, methyl jasmonate, methyl *epi*-jasmonate (*E*)-isoeugenol
- Uva; methyl salicylate, (*E*,*Z*)-2,6-nonadienal

Downloaded by CORNELL UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch002

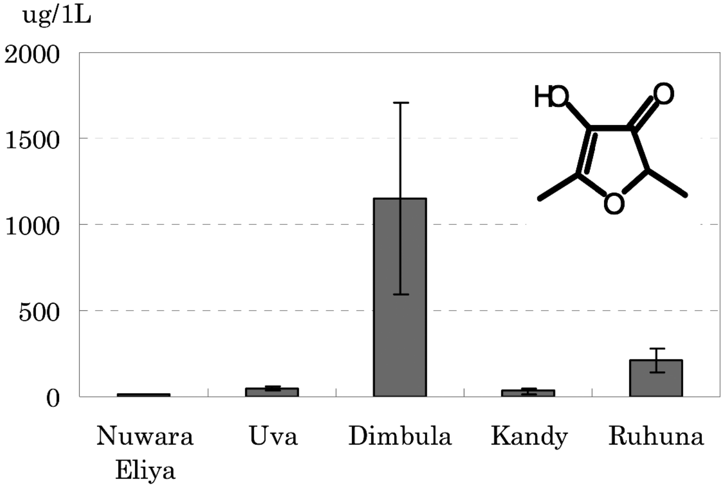


Figure 3. The concentrations of 4HDMF (Furaneol).

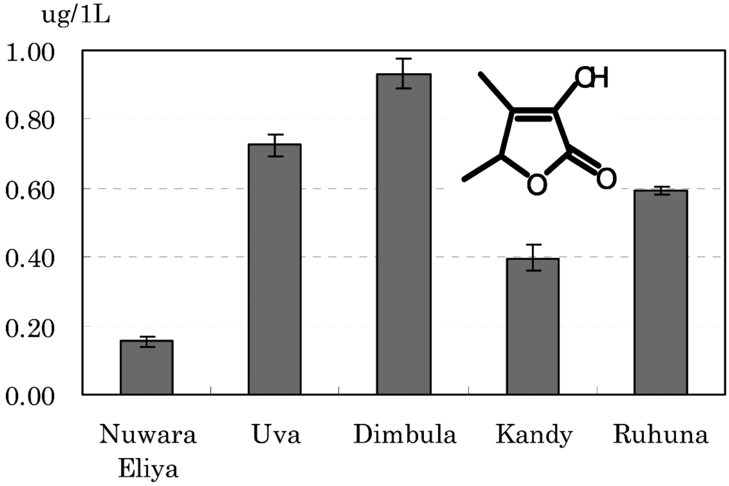


Figure 4. The concentrations of 3HDMF (Sotolon).

- Dimbula; methional, alkylpyrazines
- Ruhuna; acetylpyrazine, alkylpyrazines, methional
- Kandy; raspberry ketone, (*E*,*Z*)-2,6-nonadienal, (*E*,*E*)-2,4-nonadienal

Table III shows the ratios of enantiomers of linalool, γ-nonalactone (nonan-1,4-olide), and γ-decalactone (decan-1,4-olide). Linalool was determined to occur as (*R*)- and (*S*)-linalool in a ratio of about 60:40. All teas contained more (*R*)-linalool, which has lower odor threshold and woody lavender floral note (*13*, *14*). It might suggest that the (*R*)-linalool contributes to the aroma of all the teas, but especially in Nuwara Eliya and Uva which had a high FD-factor for linalool. Thus the sensory character of (*R*)-linalool affects the Nuwara Eliya and Uva flavors.

Figure 3 shows the comparison of the quantities of 4HDMF in a 1L infusion by the standard addition method. The quantities of 4HDMF were wide-ranging. Dimbula that has the highest concentration of over 1,200μg followed by Ruhuna at 210μg, while Nuwara Eliya, Kandy and Uva contained only 11-31μg in the infusion. The difference in the amounts of 4HDMF seemed to contribute to the overall characteristics of the aromas of five types of teas, as Dimbula and Ruhuna had higher FD factors.

Figure 4 shows the comparison of the quantities of 3HDMF in a 1L infusion by SIDA. The concentrations of 3HDMF in all the teas were all about the same level of sub μg. Amongst the teas, Dimbula contained the highest concentration of 3HDMF and 4HDMF.

The quantitative results of 4HDMF and 3HDMF support the differences of the sensory that Dimbula had the sweetest note, Kandy and Nuwara Eliya had lesser sweet notes.

Downloaded by CORNELL UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch002

Conclusion

Black tea aromas have been widely studied for long time (*2–10*), (*15–22*). This is the first time that the five types of Sri Lanka teas were analyzed using the same extraction method (brewed extract) and instrumental analysis. As a result, it was possible to clarify the common key aroma compounds in all the teas and the characteristics aroma compounds in each tea.

It is interesting that the aroma characters of the teas produced in various provinces of the small island of Sri Lanka were so different. The differences of aroma characters were presumed to come from the procedures of tea cultivation, tea production and so on. As the results were for single sets of teas in single year, variations may occur otherwise. Further studies that reveal the relationship between the aroma differences and tea cultivation or tea production are expected.

References

1. Iwabuchi, T. *Kocha-Jiten (Dictionary of Black Tea)*; Shinsei Publishing Co., Ltd.: Tokyo, 2006.
2. Kato, M.; Yano, T.; Komatsu, M.; Mori, M.; Hara, Y. A Study of the Flavor of Black Tea Produced in Indonesia (Celebes). *Nippon Shokuhin Kogyo Gakkaishi* **1993**, *40* (2), 133–137.
3. Kobayashi, A. Relationship between Aroma Analysis and Flavor Evaluation. *ILSI (International Life Science Institute Japan)* **1997**, *52*, 56–65.
4. Kumazawa, K.; Matsuda, H.; Nishimura, O.; Kato, T. Identification of Potent Aroma Components in Brewed Black Teas. *Nippon Shokuhin Kogyo Gakkaishi* **1998**, *45* (12), 728–735.
5. Yamanishi, T. Aroma of Black Tea. *Koryo* **2002**, *214*, 113–120.
6. Imayoshi, Y.; Zhong, X.; Iwabuchi, H. Study of the Volatiles of Black Tea. *Koryo* **2003**, *217*, 145–150.

Downloaded by CORNELL UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch002

7. Fernando, V.; Roberts, G. R. The Effect of Process Parameters on Seasonal Development of Flavour in Black Tea. *J. Sci. Food Agric.* **1984**, *35*, 71–76.
8. Yamanishi, T.; Kobayashi, A.; Silva, D.; Botheju, J. W. S. Aroma Formation of Sri Lankan Black Tea. *Dev. Food Sci.* **1990**, *24*, 169–177.
9. Kumazawa, K.; Wada, T.; Matsuda, H. Characterization of Epoxydecenal Isomers as Potent Odorants in Black Tea (Dimbula) Infusion. *J. Agric. Food Chem.* **2006**, *54*, 4795–4781.
10. Kawakami, M.; Kobayashi, A.; Kato, K. Volatile Constituents of Rooibos Tea (*Aspalathus linearis*) as Affected by Extraction Process. *J. Agric. Food Chem.* **1993**, *41*, 633–636.
11. Sulser, H.; Habegger, M.; Büchi, W. Synthese und Geschmackspürufungen von 3,4-Disubstituerten 2-Hydroxy-2-buten-1,4-oliden. *Z. Lebensm. Unters. Forsch.* **1972**, *148*, 215–221.
12. Blank, I. ; Schieberle, P. ; Grosch, W. Quantification of the Flavour Compounds 3-Hydroxy-4,5-dimethyl-2(5H)-furanone and 5-Ethyl-3-hydroxy-4-methyl-2(5H)-furanone by Stable Isotope Dilution Assay. *Progress in Flavour Studies*, Proceedings of the International Conference Wurzburg, Germany, September 30−October 2, 1992; 1993, pp 103−109.
13. Boonbumrung, S.; Tamura, H.; Mookdasanit, J.; Nakamoto, H.; Ishihara, M.; Yoshizawa, T.; Varanyanond, W. Characteristic Aroma Components of the Volatile Oil of Yellow Kaew Mango Fruits Determined by Limited Odor Unit Method. *Food Sci. Tech. Res.* **2001**, *7*, 200–206.
14. Bonnländer, B.; Cappuccio, R.; Liverani, F. G.; Winterhalter, P. Analysis of Enantiomeric Linalool Ratio in Green and Roasted Coffee. *Flav. Frag. J.* **2006**, *21* (4), 637–641.
15. Schuh, C.; Schieberle, P. Characterization of the Key Aroma Compounds in the Beverage Prepared from Darjeeling Black Tea: Quantitative Differences between Tea leaves and Infusion. *J .Agric. Food Chem.* **2006**, *54*, 916–924.
16. Kawakami, M.; Ganguly, S. N.; Banerjee, J.; Kobayashi, A. Aroma Composition of Oolong Tea and Black Tea by Brewed Extraction Method and Characterizing Compounds of Darjeeling Tea Aroma. *J. Agric. Food Chem.* **1995**, *43*, 200–207.
17. Wang, D.; Ando, K.; Morita, K.; Kubota, K.; Kobayashi, A. Optical Isomers of Linalool and Linalool oxides in Tea Aroma. *Biosci. Biotech. Biochem.* **1994**, *58* (11), 2050–2053.
18. Shiota, Y. Extraction and Analysis Technique of Minor Component. *Gekkan Food Chem.* **1992**, *12*, 37–40.
19. Shigematsu, Y.; Shimoda, M.; Osashima, Y. Comparison of the Odor Concentrates of Black Tea. *Nippon Shokuhin Kogyo Gakkaishi* **1994**, *41* (11), 768–777.
20. Sanderson, G. W.; Graham, H. N. On the Formation of Black Tea Aroma. *J. Agric. Food Chem.* **1973**, *21* (4), 576–585.
21. Takeo, T. Effect of the Withering Process on Volatile Compound Formation During Black Tea Manufacture. *J. Sci. Food Agric.* **1984**, *35*, 84–87.
22. Robertson, A.; Hall, M. N. A Critical Investigation into the Flavognost Method for Theaflavin Analysis in Black Tea. *Food Chem.* **1989**, *34*, 57–70.

Downloaded by UNIV OF DELAWARE MORRIS LIB on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch003

Chapter 3

A Non-Volatile Study of Teas Using Modern Analytical and Sensory Techniques

Ying Yang,* Jerry Kowalczyk, and Laurence Trinnaman

**International Flavors & Fragrances Inc. Research and Development,
1515 Highway 36, Union Beach, NJ 07735, USA
*ying.yang@iff.com**

The differentiation of a variety of teas was carried out based on the contents of their taste-active components. Forty-four taste active components including the following five groups: amino acids (twenty naturally-occurring amino acids, theanine and ornithine), organic acids, sugars, phenolic compounds and purine alkaloids were determined by liquid chromatography in tea infusions. To further bridge the gap between pure analytical chemistry and human taste perception, the dose-over-threshold (DOT) factors were calculated on the basis of a dose/threshold relationship for these taste-active components, and thus taste spider charts were constructed. Analyses of these taste spider charts for different tea infusions suggested that it not only provides an easy and unbiased comparison and classification for multiple tea samples in taste, but also is able to narrow the number of key taste compounds from forty-four to thirteen as chemometric descriptors for classification purposes of different tea varieties. Thus, an analytical method was developed to quantify these taste active components by NMR spectroscopy, and in conjunction with the sensory spider charts, could lead to a more rapid and unbiased assessment for the taste of a variety of teas.

In Flavors in Noncarbonated Beverages; Da Costa, N., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

Downloaded by UNIV OF DELAWARE MORRIS LIB on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch003

Introduction

For many centuries, the freshly prepared infusion of the dried leaves and buds of the plant *Camellia sinensis* has been consumed by humans as the most popular drink after water (*1*). Among many different tea varieties with different external qualities and inner qualities due to chemical diversities (*2*), three major tea types were categorized as green (unfermented), black (fermented) and oolong (semi-fermented). Taste quality is one of the key criteria used by the master tea tasters to describe the quality of tea infusions. Therefore, developing an analytical method to assess the tea taste could provide an unbiased and effective tool for tea quality control and greatly assist tea flavor creation.

The most important non-volatile chemical components that influence the taste of tea infusion are polyphenols, flavonols, caffeine, sugars, organic acids, amino acids (*3*) ornithine and theanine (Figure 1). Much research has been reported on the non-volatile chemical composition, key taste compounds responsible for different taste attributes, and correlation between chemical composition and tea taste (*4*). The metal ion content, catechins, purine alkaloids, and volatile compounds have been used as chemical parameters to differentiate between tea varieties (*5*, *6*). Including the entire non-volatile chemical composition as main parameters to differentiate different tea varieties has not been previously reported. The objective of this study is to create an analytical tool that can translate the chemical composition into a taste profile for the purpose of differentiating teas.

Initially four freshly prepared tea infusions including two black and two green teas were chosen to quantify taste active compounds. These four tea samples were rated using dose-over-threshold (DOT) factors, and these factors used to construct taste spider charts for classification purposes.

In addition, to being able to rapidly provide analytical data for a quick assessment of a tea variety, a ^{1}H-NMR method was developed due to its ability to display the entire organic chemical mixture in a single spectrum. The goal of the study was to examine the potential of this NMR method for the analysis of a complex ingredient, and to correlate the amount of taste active components with the quality of tea variety by using the information extracted from ^{1}H-NMR spectra. The comparison of this method with routine chromatographic methods for analyzing taste active compounds such as amino acids, organic acids, sugars, phenolic compounds was conducted in this study.

Experimental

Sample Preparation for LC

For amino acid analysis, 6.0g of each type of dried tea leaf was steeped in 36.0g boiling water for 3 min. The solution was rapidly cooled in an ice bath. The supernatant was filtered with a 0.45µm nylon filter prior to LC analysis. For other analyses, 2.0g of each type of dried tea leaf was extracted in 36.0g boiling water for 3 min. Two types of black tea (Lipton and Keemun black tea) and two types

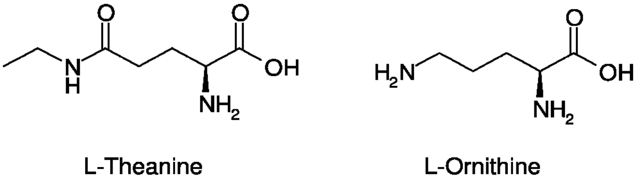


Figure 1. Amino acid related analogues in tea.

of green tea ("Summer" Long Jin and "Spring" Long Jin green tea) were selected for this study.

LC Conditions

Free amino acids, LC-MS: Finnigan Surveyor system/ LXQ-34000, Column: TSK-Gel Amide 80 (250 x 4.6mm), Mobile Phase: Eluent A: 40% aqueous ammonium acetate buffer (6.5mM) in 60% acetonitrile; Eluent B: 10% aqueous ammonium acetate buffer (6.5 mM) in 90% acetonitrile. Free Organic acids, HPLC-UV Agilent Technologies 1100LC Column: BIORAD 300x7 Amines XPX-87H, UV @ 210nm, Mobile Phase: Eluent 0.005 M Sulfuric acid – isocratic, Temperature: 55°C, Flow: 0.6 ml/min, gradient run for 30min. standards (Sigma). Free Sugars, HPLC-UV Agilent Technologies 1100LC Column: Prevail carbohydrate ES (250 x 4.6mm), 5µm, ELSD detector, Mobile Phase: Acetonitrile: Water (75:25) Flow rate: 1.0 mL/min, gradient run for 45 min. Phenolics and purine alkaloids, HPLC-UV Agilent Technologies 1100LC Column: Synergie RP Max 80 (250 x 4.6 mm), 5 um. Mobile phase: Eluent: A= 0.1% formic acid in water, B = 0.1% formic acid in acetonitrile, gradient run for 60 min.

IC Conditions

Cations, DIONEX BIO IC, Column: CS14. Mobile Phase: A: 18MΩ water; B: 20mM Methanesulfonic acid 70% w/w in water.

Sample Preparation and NMR Measurement

Tea sample (100.0mg; finely ground using a coffee grinder) was stirred with 1200µL of extraction solvent consisting of D_2O with 500ppm sodium 3-(trimethylsilyl) propionate-d4 as a reference at 70°C for 10 min, allowed to cool, and centrifuged at 10,000rpm for 10 min. Each NMR sample consisted of 600 µL of the supernatant.

^{1}H-NMR (500 MHz Varian). D_2O. 256 scans, an acquisition time of 2 sec, and a recycle delay of 8 sec per scan. Water suppression during the recycle delay. Each acquisition was for 42 min.

Downloaded by UNIV OF DELAWARE MORRIS LIB on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch003

Downloaded by UNIV OF DELAWARE MORRIS LIB on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch003

Table I. The contents of taste active compounds in four tea samples*

	Lipton tea	*Keemun tea*	*Spring tea*	*Long Jin tea*
Amino Acid (%)				
Phenylalanine	0.02	0.02	0.02	0.01
Leucine	0.02	0.01	0.01	0.02
isoLeucine	0.02	0.01	0.01	0.01
Tyrosine	0.02	0.02	0.01	0.01
Valine	0.03	0.03	0.02	0.02
Proline	0.01	0.01	0.01	0.01
Alanine	0.03	0.02	0.02	0.03
Threonine	0.02	0.02	0.03	0.03
Glycine	0.01	0.01	0.01	0.01
Serine	0.05	0.03	0.06	0.06
Glutamic acid	0.11	0.06	0.23	0.24
Arginine	0.01	0.02	0.16	0.12
Lysine	0.01	0.01	0.01	0.01
Aspartic acid	0.10	0.05	0.18	0.21
Tryptophan	0.02	0.02	0.01	0.01
Theanine	0.24	0.13	0.30	0.35
γ-Aminobutyric acid	0.03	0.03	0.03	0.04
Glutamine	0.06	0.04	0.22	0.15
Asparagine	0.06	0.05	0.04	0.07
Ornithine	0.01	0.01	0.01	0.01
Sugars (%)				
Fructose	0.84	0.52	0.29	nd
Glucose	0.96	0.48	0.09	0.11
Sucrose	1.43	nd	0.69	0.58
Maltose	nd	nd	nd	nd
Organic acids (%)				
Oxalic acid	3.79	4.24	1.55	1.50
Citric acid	0.34	0.27	0.15	0.24
Malic acid	0.43	0.32	0.22	0.24
Succinic acid	0.04	0.06	0.05	0.07
Pryoglutamic acid	0.05	0.21	0.38	0.14
Quinic acid	1.49	1.58	0.92	0.90
Cations (%)				
Sodium	0.07	0.08	0.05	0.05
Ammonium	nd	nd	0.24	0.19
Potassium	1.56	1.48	1.29	1.35
Magnesium	0.12	0.08	0.04	0.05
Calcium	0.02	0.01	0.01	0.02
Polyphenolics (%)				

Continued on next page.

Table I. (Continued). The contents of taste active compounds in four tea samples*

	Lipton tea	*Keemun tea*	*Spring tea*	*Long Jin tea*
5-Galloylquinic acid	0.29	0.29	1.93	1.76
Theobromine	0.19	0.19	0.18	0.11
Gallocatechin	0.15	0.15	0.93	0.60
Catechin	nd	0.00	0.10	nd
Caffeine	2.18	2.18	1.91	1.86
Epicatechin	0.09	0.09	0.34	0.23
Epigallocatechin gallate	nd	nd	1.16	0.52
4-p-Coumaroylquinic acid	0.06	0.06	0.43	0.47
Epicatechin-3-gallate	0.06	0.06	0.77	0.41
Total (%)	15.1	12.9	15.1	12.8

* The contents of the compounds underlined were calculated by using the calibration curve of epi-catechin due to their unavailability.

Downloaded by UNIV OF DELAWARE MORRIS LIB on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch003

Results and Discussion

Part I. From Analytical to Taste Spider Charts

Analytical Results

A pair of black teas and a pair of green teas were selected for this study. Lipton is an American black tea in a bag form, and Keemun is a popular Chinese black tea. Spring and Long Jin are unfermented green teas, coming from the same plant species. The difference is that Spring tea is picked in March, while Long Jin tea is picked in the summer.

These four tea samples were extracted with hot water and the supernatants were directly analyzed by five different LC and IC methods to give five different groups of taste active chemicals (Table I). The amino acid profile contained twenty naturally occurring amino acids and some unique tea amino acids such as theanine, ornithine, γ-aminobutyric acid and others. Glutamic acid, arginine, aspartic acid, theanine, and glutamine are the major amino acids in green tea. Overall, the content of amino acids in tea samples is very low, especially in black tea. The low content of amino acids in black tea may be due to degradation during the fermentation process. The sugar profile in these four tea samples was inversely proportional to that of the amino acid profile. This suggested that fermentation released more fructose and glucose from glycosides in black tea. Oxalic acid and quinic acid were two major organic acids in all of four tea samples. In black tea, the contents of oxalic acid and quinic acid were much higher than those in green tea. Again, the polyphenolic profile was inversely proportional to the organic acid profile in these two types of tea. Polyphenolic compounds such as catechin, epi-catechin, epi-galocatechin gallate (EGCG) were rich in the green teas especially in early picked spring tea. For the mineral profile, no major differences were found between these

Downloaded by UNIV OF DELAWARE MORRIS LIB on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch003

Table II. Reported data taste thresholds of taste active compounds (*8*, *9*)

	Compound	*Threshold (µmol/L)*
Salty	Sodium	7500
	Potassium	15000
	Magnesium	4000
	Chloride	7500
Astringent	Catechin	410
	Epicatechin-3-gallate	260
	Epigallocatechin	520
	Epicatechin	540
	Catechin-3-gallate	930
Bitter	Theobromine	800
	Caffeine	750
	isoLeucine	11000
	Leucine	12000
	Phenylalanine	58000
	Tyrosine	5000
	Valine	21000
Sour	Succinic acid	900
	Oxalic acid	5600
	Malic acid	3700
	Citric acid	2600
Umami	Aspartic acid	7500
	Glutamic acid	200
	5'-AMP	2000
	5'-GMP	300
	Succinic acid	900
Sweet	Glucose	90000
	Sucrose	24000
	Fructose	52000
	Serine	30000
	Alanine	8000
	Glycine	30000
	Ornithine	3500
	Proline	26000
	Threonine	40000

two types of tea. Potassium content stood out among all the cations, as it was the major mineral that provided a salty impact. Clearly, these five groups of taste active chemicals could be used as good chemical descriptors to differentiate between the tea varieties.

Downloaded by UNIV OF DELAWARE MORRIS LIB on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch003

A Link between the Analytical Data and Tea Taste

To more easily display and comprehend the results of these multiple compound analyses it was easier to create a simple, visual chart to differentiate and characterize the tea tastes. Several research groups have already studied the taste of non-volatile chemicals and determined their taste threshold levels. Thus, all of this published data was compiled into six taste groups: salty, astringent, bitter, sweet, sour, and umami (Table II).

These six group's taste threshold data covered most of non-volatile chemicals found in our tea study. As listed in Table II, the salty taste comes mainly from minerals. Most of polyphenolic chemicals contribute to the astringent taste at different levels. The bitter taste is contributed by a different group of chemicals. Alkaloids, such as theobromine and caffeine have prominent bitter taste attributes. Most of hydrophobic amino acids are also associated with a bitter taste, but they have relatively high thresholds and relatively low contents in tea. Interestingly, succinic acid not only contributes to the sour taste but is also associated with umami taste effects. Amino acids such as glutamic acid, aspartic acid, and theanine were found to be the major contributors to the umami taste. Ribonucleotides such as 5'-AMP, 5'-IMP and 5'-GMP are known to be mainly umami-enhancing. The sweet taste attributes not only come from sugars, but is also contributed to by amino acids such as serine, alanine, glycine and others.

Differentiation of Teas

The concentrations of forty-four chemicals from tea analysis were divided by their corresponding chemical taste threshold values. These ratio values were grouped together for the same taste attribute, resulting in four taste spider charts (Figure 2).

Lipton and Keemun black fermented teas have very similar taste profiles. The green teas, Spring and Long Jin, show different taste profiles from black tea. They are more astringent and umami in taste than the black teas. Between these two green teas, the Spring tea is more astringent than the Long Jin tea. This reflects a higher content of polyphenolic chemicals in the younger leaves of Spring tea.

From the results of this study it can be concluded that the analytical taste spider charts are a useful tool for an unbiased comparison and classification for multiple tea samples in taste.

We also examined the entire chemical composition from forty-four chemicals and their contribution to the tea taste and found thirteen compounds were the key players for the taste of green and black teas; all are found in Table I. Other chemicals, either due to their trace levels, or very high taste thresholds, had little impact on the overall taste.

Downloaded by UNIV OF DELAWARE MORRIS LIB on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch003

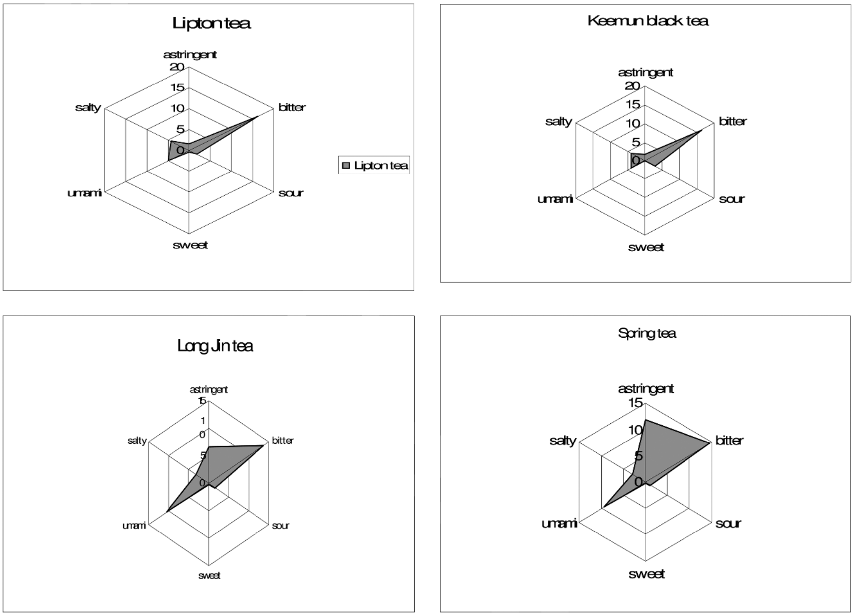


Figure 2. Taste profiles predicted from analytical data.

Part II. NMR Method Development – A Quick Method for Tea Quality Assessment

To accommodate the requirements from fast pace industry, a quick NMR method was developed for tea quality assessment. Proton NMR with presaturate water suppression was used to acquire ^{1}H-NMR data. For each tea extract preparation, we mimicked the normal tea preparation procedure to produce a tea extract close to that of the tea drink. For quantification purposes, a fixed amount of internal standard was introduced to each extract.

Eight taste-active compounds were clearly assigned based on NMR data from the Biorad NMR database and a reference (*7*), as shown in Figure 3. The selected peaks were integrated and normalized with the internal standard value to give a ratio as shown in Figure 4.

From these relative ratio values of the compounds of interest among these nine tea samples, a concentration trend was observed. The content profiles of caffeine calculated from two different chemical shift peaks (3.42ppm and 7.7ppm) consistent with each other, indicated the reproducibility of this method. This reproducibility also can be seen in the content profiles of theanine. In addition, in green tea J and green tea D, which were both prepared from the same tea sample, the relative contents of most of chemicals matched each other. This suggested a good reproducibility of this method. Thus, the relative content profiles (Figure 3) of eight taste active compounds among these nine tea samples were used to correlate the grades of these teas based on their prices. One example was shown in Figure 5.

Downloaded by UNIV OF DELAWARE MORRIS LIB on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch003

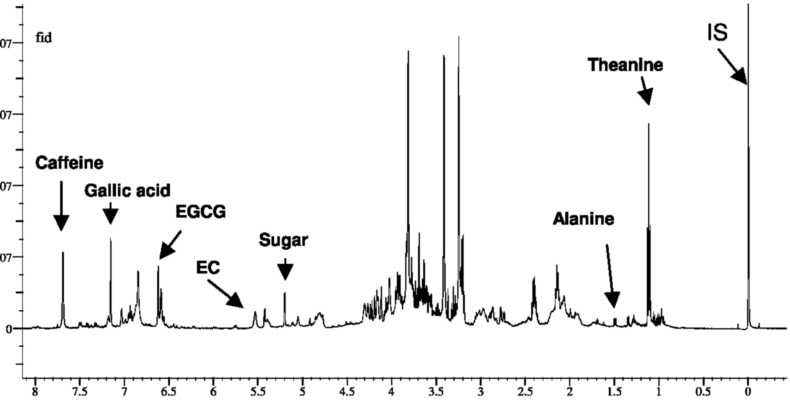


Figure 3. The ^1H NMR spectrum of Long Jin green tea A.

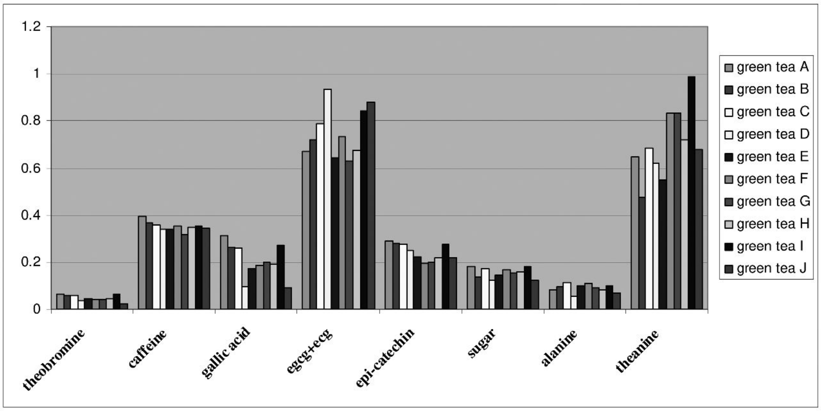


Figure 4. The relative ratio of eight taste active compounds in nine different grade Long Jin tea samples by ^1H NMR measurement.

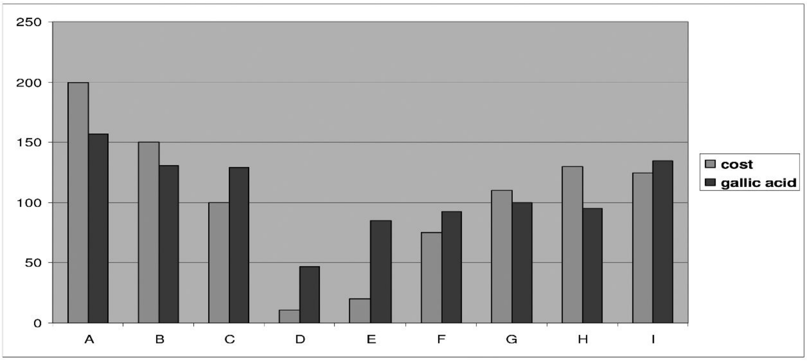


Figure 5. Price versus relative contents of gallic acid in nine Long Jin teas.

It was observed that gallic acid was present at higher concentrations in high quality teas. The correlation of the relative content profiles of nine compounds from NMR data and price of these nine tea samples indicated gallic acid as a useful marker for the quality control of Long Jin green tea.

Thus, this method can be used to simultaneously analyze the polyphenolics, amino acids, organic acids and sugars from a single green tea extract. It reduced analytical time from fives days (five liquid-chromatographic methods) to half a day (one 1H-NMR method). In addition the sample preparation and instrument setup were much simpler than those for chromatographic methods. However, due to overlapping of 1H-NMR signals, a limited number of taste active compounds were applicable for this method.

Downloaded by UNIV OF DELAWARE MORRIS LIB on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch003

Conclusion

The spider charts derived from analytical data were tools for an easy and unbiased comparison and classification of multiple tea samples in taste.

The correlation between initial NMR data and the quality of nine Long Jin tea samples suggested a promising QC method for a quick screening of teas for their taste quality.

References

1. Weisburger, J. H. Tea and health: A historical perspective. *Cancer Lett.* **1997**, *114*, 315–317.
2. Chen, Q.; Zhao, J.; Fang, C. H.; Wang, D. Feasibility study on identification of green, black and Oolong teas using near-infrared reflectance spectroscopy based on support vector machine (SVM). *Spectrochim. Acta A* **2007**, *66*, 568–574.
3. Seetohul, L. N.; Islam, M.; O'Hare, W. T.; Ali, Z. Discrimination of teas based on total luminescence spectroscopy and pattern recognition. *J. Sci. Food Agric.* **2006**, *86*, 2092–2098.
4. Alcázar, A.; Ballesteros, O.; Jurado, J. M.; Pablos, F.; Martín, M. J.; Vilches, J. L.; Navalón, A. Differentiation of Green, White, Black, Oolong, and Pu-erh Teas According to their Free Amino Acids Content. *J. Agric. Food Chem.* **2007**, *55* (15), 5960–5965.
5. Moreda-Pineiro, A.; Fisher, A.; Hill, S. J. The classification of tea according to region of origin using pattern recognition techniques and trace metal data. *J. Food Compos. Anal.* **2003**, *16*, 195–212.
6. Fernandez, P. L.; Martin, M. J.; Gonzalez, A. G.; Pablos, F. HPLC determination of catechins and caffeine in tea. Differentiation of green, black and instant teas. *Analyst* **2000**, *125*, 421–425.
7. Le Gall, G; Colquhoun, I. J.; Defernez, M. Metabolite profiling using 1H NMR spectroscopy for quality assessment of green tea, Camellia sinensis (L.). *J. Agric. Food Chem.* **2004**, *52* (4), 692–700.

8. Kaneko, S.; Kumazawa, K.; Matsuda, H.; Henze, A.; Hofmann, T. Molecular and Sensory Studies on the Umami Taste of Japanese Green Tea. *J. Agric. Food Chem.* **2006**, *54* (7), 2688–2694.
9. Scharbert, S.; Hofmann, T. Molecular Definition of Black Tea Taste by Means of Quantitative Studies, Taste Reconstitution, and Omission Experiments. *J. Agric. Food Chem.* **2005**, *53* (13), 5377–5384.

Downloaded by UNIV OF DELAWARE MORRIS LIB on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch003

Downloaded by OHIO STATE UNIV LIBRARIES on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch004

Chapter 4

Functional Contribution of Polyphenols in Black Tea

Yu Wang and Chi-Tang Ho*

Department of Food Science, Rutgers University, 65 Dudley Road New Brunswick, NJ 08901-8520, USA
***ho@aesop.rutgers.edu**

Tea is one of the most popular beverages consumed worldwide. Freshly harvested tea leaves are converted into green, oolong and black teas during processing. More than 75% of the world tea products are black tea, and polyphenols in black tea contribute to the majority of its reported biofunctions. In this paper we review the chemistry properties of polyphenols and bio-function properties including taste, anti-inflammation and anti-cancer. In the course of studies on the oxidation mechanisms of tea polyphenols, a new type of a tea pigment, theadibenzotropolone A, together with theaflavin 3-gallate were formed in the reaction of (-)-epicatechin (EC) and (-)-epigallocatechin gallate (EGCG). Theaflavins and flavon-3-ol glycosides have been shown to give an astringent perception with low threshold. Theaflavins have also demonstrated strong capabilities of anti-inflammation and anti-cancer via the NF-*k*B and MAPKs pathways, respectively.

Introduction

Tea, one of the most popular beverages in the world, has been consumed for thousands of years. Tea is produced by brewing the dried leaves and buds of the plant *Camellia sinensis*, which was first cultivated in China and then Japan. With the opening of ocean routes to the East by European traders during the fifteenth to seventeenth centuries, commercial cultivation gradually expanded to Indonesia and then to the Indian subcontinent, including what is now Sri

In Flavors in Noncarbonated Beverages; Da Costa, N., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

Lanka (*1*). In 1657, tea first reached Britain. Tea is now second only to water in worldwide consumption. Annual production of about 1.8 million tons of dried leaves provides world per capita consumption of 40 liters of beverages (*2*). Tea is generally classified into three types based on different processing methods, specifically green tea (non-fermented tea), oolong tea (partially fermented tea), and black tea (fully fermented tea) (*3*).

Many studies have shown that a relationship exists between the consumption of tea and potential disease prevention properties which may be due to high polyphenol content (*4*, *5*). Polyphenols are secondary metabolites of plants that are used in their defense system against severe environments such as ultraviolet radiation and pathogens. These compounds are generally classified into flavonoids, phenolic acids, lignans and stilbenes. Flavonoids, the most ubiquitous polyphenols, are benzo-γ-pyrone derivatives consisting of phenolic and pyrane rings, and are classified into flavanols, flavones, flavonols, flavanones, isoflavones and anthocyanidins. Phenolic acids are divided into two subclasses: derivatives of benzoic acid and derivatives of cinnamic acid.

The major polyphenols in tea are flavonoids, particularly flavanols (i.e. catechins) and phenolic acids. A green tea infusion (200ml) may contain up to 200mg of catechins . Black tea contains very few flavanol monomers which are easily oxidized into dimers (theaflavins) and polymers (thearubigins). Tea is also an important source of gallic acid, which is a hydroxybenzoic acid. Chinese Pu-er teas contain the highest level of gallic acid (~15 g/kg dry weight) compared to other types of tea (*7*).

This paper mainly reviews the polyphenols in black tea including their formation process, as well as their properties of taste and anti-diseases.

Downloaded by OHIO STATE UNIV LIBRARIES on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch004

Polyphenols in Black Tea

The major polyphenols in tea are flavonoids and phenolic acids. Due to differences in manufacturing, the types of polyphenols in the three major types of teas, green tea, black tea, and oolong tea are very different.

More than 75% of world tea production is black tea. The steps involved in the processing of black tea include withering, leaf disruption, fermentation, drying and grading. All steps are designed to achieve optimal oxidation of tea catechins and produce tea products with good flavor and color. However, in the processing of green tea, the tea leaves are either first steamed, in the case of Japanese green tea “sen-cha” or pan-fried, in the case of Chinese green tea (*8*). These heat treatments inactivate enzymes in the tea leaves. Following heat treatment, tea leaves are subjected to subsequent rolling and drying processes. Oolong tea is prepared by frying the leaves after rolling to terminate the oxidation process. It is only partially oxidized and retains a considerable amount of the original polyphenols (*9*).

Tea polyphenols, also knows as catechins, account for 30% to 42% of water-soluble solids in brewed green tea. There are four major tea catechins: (T)-epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC) and (-)-epigallocatechin-3-gallate (EGCG) (*12*). The structures of these catechins are shown in Figure 1. Epicatechin and epigallocatechin were isolated and identified in green tea in early 1930 (*10*). In the late 1940's Bradfield and his coworkers identified and quantified catechin, epicatechin, gallocatechin, epicatechin gallate and gallocatechin gallate in a Ceylon green tea (*11*). The most abundant compound, gallocatechin gallate was later proved to be epigallocatechin gallate (*12*).

Most of the important chemical changes that take place in the black tea occur during the fermentation process. Polyphenol oxidase and peroxidase, which are responsible for the oxidation of flavonols, oxidizes pyrogallol and catechol to their *o*-quinones. Further chemical reactions then lead to various oxidation products. During fermentation, the characteristic black tea polyphenols, theaflavins and thearubigins, are generated. Four major theaflavins have been identified from black tea, including theaflavin (TF), theaflavin-3-gallate (TF3G), theaflavin-3′-gallates (TF3'G), and theaflavin-3,3′-digallate(TFDG) (Figure 2). The proposed mechanism of theaflavin formation is shown in Figure 3. Thearubigin is known as a heterogeneous mixture of pigments (*13*).

Roberts *et al* (*14*) reported fermented black tea contained polyphenolic pigments that are not found in unprocessed tea leaves. They designated the brown acidic pigments and the yellow neutral pigments as thearubigins and theaflavins, respectively. They suggested that theaflavin was the coupling oxidation product of EGC and EGCG, having benzotropolone structure. Later, Takino *et al* (*15*) corrected the structure of theaflavin and revealed that theaflavin was the coupling oxidation product of EC and EGC. Theaflavin formation was confirmed by enzymatic oxidation with crude tea polyphenol oxidase and chemical oxidation with potassium ferricyanide. It was clear that theaflavins are produced by enzymatic co-oxidation of appropriate pairs of catechins, one having a *vic*-trihydroxy structure and the other having an *ortho*-dihydroxy group, followed by condensation.

Takino *et al* (*15*) proposed a theaflavin formation mechanism based on the formation of purpurogallin from pyrogallol. The results suggested the possibility of the existence of similar pigments from the other pairs of flavanols. Bryce *et al* (*16*) successfully isolated three other pigments from black tea and confirmed their parent flavanols using chemical oxidation methods. One of them, named TF1, was identical to the theaflavin reported by Roberts (*14*). TF2 was identified as a mixture of TF2A and TF2B, which were produced by chemical oxidation from EGCG and EC (TF2A) and EGC and ECG (TF2B), respectively. TF3 was an identical compound that was isolated from the ferricyanide oxidation of EGCG and ECG. They identified TF4 as an oxidation product from gallic acid and EC. Later, Collier *et al* (*17*) confirmed the presence of four theaflavins in black tea and also the presence of epitheaflavic acid and epitheaflavic acid gallate, which are identical oxidative products from the coupling of EC and gallic acid, and the coupling of ECG and gallic acid, respectively. Isotheaflavin, which was reported by Coxon *et al* (*18*, *19*) was also identified to be present in black tea and was confirmed to be an oxidative coupling product of EGC and catechin. Compared to theaflavins, epitheaflavic acid, theaflavic acid and epitheaflavic acid gallate occur at a much lower concentration. The relative proportions of the theaflavins in black tea were theaflavin (18%), theaflavin-3-gallate (18%),

Downloaded by OHIO STATE UNIV LIBRARIES on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch004

Downloaded by OHIO STATE UNIV LIBRARIES on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch004

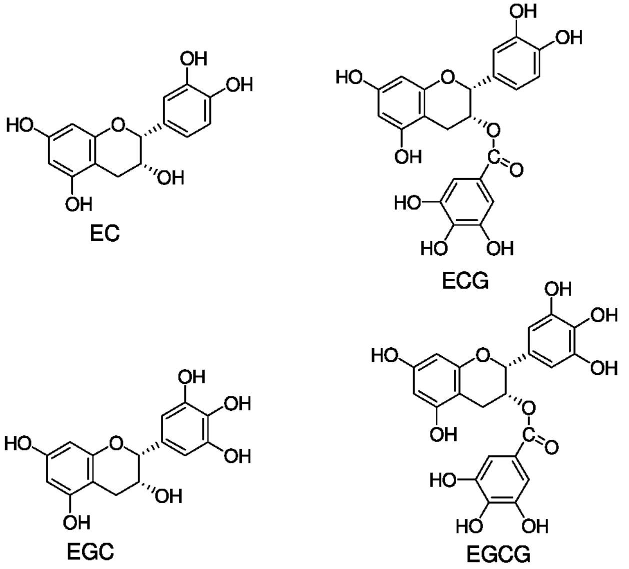
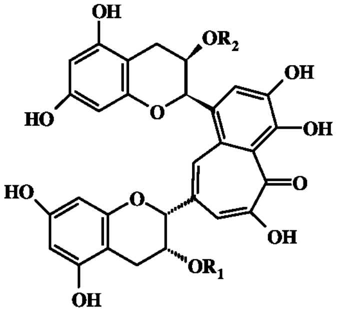


Figure 1. Polyphenols in green tea.



		R_1	R_2
Theaflavin	**TF**	**H**	**H**
Theaflavin 3-gallate	**TF3G**	**Gallate**	**H**
Theaflavin 3'-gallate	**TF3'G**	**H**	**Gallate**
Theaflavin 3,3'-gallate	**TFDG**	**Gallate**	**Gallate**

Figure 2. Structures of theaflavins.

theaflavin-3’-gallate (20%), and theaflavin-3,3’-digallate (40%) and theaflavic acids, along with isotheaflavin, were approximately 4% (*20*). Several other minor pigments have also been reported from black tea, such as theaflavate A, theaflavate B, isotheaflavin-3′-*O*-gallate and neotheaflavin-3-*O*-gallate (*21*, *22*).

Downloaded by OHIO STATE UNIV LIBRARIES on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch004

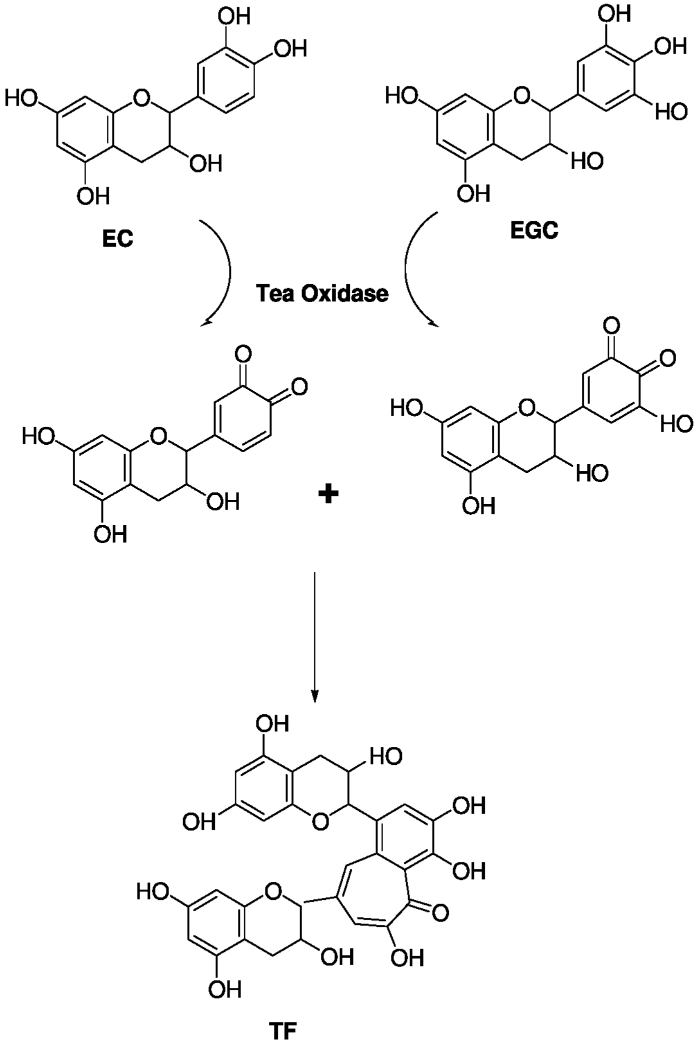


Figure 3. The proposed mechanism of theaflavin formation.

Recently, it has been reported that the galloyl ester group of theaflavin 3-gallate is as reactive as the B-ring (*vic*-trihydroxy) of EGCG or EGC and the galloyl ester group of ECG, and can further react with EC to form a new theaflavin type tea catechin trimer, theadibenzotropolone A which was characterized from the ethyl acetate fraction of black tea extract by LC/ESI-MS/MS (Figure 4) (*23*).

Thearubigins account for approximately 10-20% of the dry weight of black tea. However, because of their hot water solubility, they account for approximately 30-60% of the solids in black tea infusions (*14*). Even though thearubigins are suggested as further oxidation products of theaflavins and catechins, their formation mechanism and chemical structures are unclear.

Thearubigins have a wide range of molecular weights, ~700-40,000 daltons, and are regarded as polymeric compounds. Brown *et al* (*24*) reported that thearubigins are a polymeric mixture of proanthocyanidins containing flavonoid residues, and they proposed their formation mechanism through the creation of a C-C bond. Later, Berkowitz *et al* (*25*) suggested that the formation of epitheaflavic acid from EC and gallic acid plays an important role in the formation of thearubigins. They found that when EC was added to a tea fermentation system with epitheaflavic acid, both compounds disappeared rapidly, while thearubigin content increased. Interestingly, when it was reacted with only epitheaflavic acid in a tea fermentation system no reaction occurred. The exact chemistry of thearubigins remains unclear.

Downloaded by OHIO STATE UNIV LIBRARIES on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch004

Bio-Function Properties of Polyphenols in Black Tea

Taste

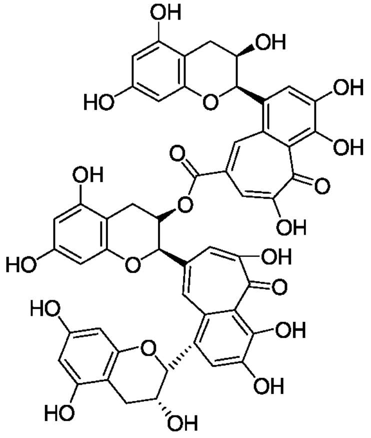
Flavor is the most important factor in determining the quality of tea. Flavor involves both taste and aroma. Polyphenols, as nonvolatile compounds have been studied from their taste qualities (bitter, sweet, umami, salty and sour) and chemosensations (astringent, cooling, hot, pungent etc.) which are important for the balance of tea taste. The major contributors to astringency and bitterness of tea are catechins and caffeine, respectively.

Several studies have revealed a relationship between the chemical composition of tea leaves and the final quality of black tea products (*26*). During the fermentation process, the catechin content of tea leaves decreases, whereas new oxidation products, such as theaflavins and thearubigins, increase. However, due to differences in redox potential of catechins, tea catechins are depleted at different rates. For example, pyrogallol (EGC, EGCG) may deplete faster than catechol (EC, ECG). Catechins have a different degree of astringency. Table I. lists the threshold levels for astringency and bitterness of catechins, theaflavins and caffeine.

In general, gallated catechins have more astringency. The theaflavins have astringent tastes and contribute to the briskness of black tea. Theaflavin-3,3′-digallate is the most astringent (6.4 times higher than theaflavin), and theaflavin monogallates rank next to digallate for astringency. Thus, theaflavin composition is regarded as an important factor affecting the color and briskness of black tea. Generally, extended fermentation times and increased fermentation temperatures are unsuitable for obtaining high quality black tea products. Robertson *et al* (*27*) reported that, according to *in vitro* oxidation experiments, high oxygen tension, low pH (pH 5.0) and low temperature (30°C) gave high yields of theaflavin formation. Black tea fermentation is a complicated mechanism that can influence the final quality of black tea, not only by catechin composition of tea leaves, but also by polyphenol oxidase activity.

However, from Table I it is clear that the concentrations of catechins and four major theaflavins cannot count for the perceived astringent taste of black tea

Downloaded by OHIO STATE UNIV LIBRARIES on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch004



theadibenzotropolone A

Figure 4. Structure of theadibenzotropolone A.

beverages alone. A group of flavon-3-ols have been shown to have the highest taste dilution factor and taste impact, compared with catechins and theaflavins (*28*). Although gallated catechins have more astringency, there is no significant difference observed from that of theaflavins. Furthermore, the type of flavon-3-ol aglycone and the type and the sequence of the individual monosaccharides are key factors influencing the astringency perception of flavon-3-ol glycosides. Besides flavonoids as tastants, phenolic acids such as gallic acid and theogallin (gallic acid esterified with quinic acid) have been shown to have an astringent perception with low threshold (*29*).

Anti-Inflammation

Inflammation may be defined as the reaction or response of the body to tissue injury or damage and is commonly characterized by four symptoms namely redness, swelling, pain and heat (*30*, *31*). Cell or tissue damage may be caused by several agents such as heat, chemicals, radiation, microbial infection, trauma, as well as certain immunological processes. To counteract the tissue damage, the body has two types of defense systems, the primary defense system (immediate such as inflammation) and the secondary defense system (controlled by the immunological system) (*32*). During this process, temporary vasoconstriction of blood vessels takes place followed by local vasodilation and increased capillary permeability which leads to redness, edema and heat. Simultaneously, leukocytes migrate to the site of injury through the process of chemotaxis to attack the antigen. These processes are referred to as acute inflammation during which various mediators, reactive oxygen species (ROS), and reactive nitrogen species (RNS) are released to help control the inflammatory process (*33*–*35*). However, if the acute inflammation persists over an extended period of time,

Downloaded by OHIO STATE UNIV LIBRARIES on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch004

Table I. Threshold Levels for Astringency and Bitterness of Catechins and Theaflavins in Black Tea

Polyphenol	*Threshold Level (mg/100 mL)* *Astringency*	*Bitterness*	*Approximate Level in a Cup of Black Tea* (mg/100mL) of Beverage*
(-)-Epicatechin	Not astringent	60	Trace
(-)-Epicatechin gallate	50	20	Trace
(-)-Epigallocatechin	Not astringent	35	Trace
(-)-Epigallocatechin gallate	60	30	16-18
(+)-Catechin	Not astringent	60	Trace
Theaflavin	80	75-100	0.6-1.2
Natural mixture of theaflavins monogallate	36	30-50	1.8-3.7
Theaflavin 3,3′-digallate	12.5	unknown	2.4-4.8
Gallic acid	Not astringent	Not bitter	3-5

due to dysregulation of various events, it can lead to the development of chronic inflammation.

The basic mediators of inflammation include cytokines, ROS/RNS, arachidonic acid (AA) metabolites, and NF-*k*B. The cytokines (peptide mediators of inflammation) are important intracellular messengers that play an important role in the inflammatory process (*36*). They have a short duration of action and perform several diverse functions; such as growth regulation, cell division, inflammation and immunity, by interacting with specific receptors present in different cells (*37*, *38*). Cytokines can be of different classes and possess both pro- and anti-inflammatory properties. The interleukins (IL) are cytokines produced by leukocytes, which are important in the inflammatory process. The important pro-inflammatory cytokines include tumor necrosis factor α (TNF α), interleukin-1 (IL-1) and interleukin-6 (IL-6). The ROS/RNS that are released during inflammation (such as hydrogen peroxide, nitric oxide and superoxide anion) have also been shown to play a major role in the process of carcinogenesis. These free radicals have been shown to damage proteins, DNA, RNA and lipids leading to mutations in several key genes involved in carcinogenesis (*39*, *40*). Exposure to ROS/RNS has been shown to increase the expression of cytokines, activate NF-*k*B, and enhance inflammation. AA is released from the membrane phospholipids catalyzed by phospholipase and is metabolized to prostaglandins and leukotrienes by cyclooxygenase (COX) and lipoxygenase (LOX), respectively (*41*). The metabolites of AA have been shown to display inflammatory properties such as the enhancement of the expression of NF-*k*B, and disorder activation and/or up-regulation of COX and LOX have been suggested in inflammatory processes (*42*, *43*). In addition, the up-regulation of oxidative metabolism of AA that produces excessive prostaglandins, leukotrienes and ROS is one the main events in the inflammatory process.

In Flavors in Noncarbonated Beverages; Da Costa, N., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

Downloaded by OHIO STATE UNIV LIBRARIES on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch004

NF-*k*B is a transcription factor (a complex of proteins) that binds to DNA and actives gene transcription, and is the central coordinator of innate and adaptive immune responses. Activated NF-κB often facilitates transcription of numerous genes, including iNOS, COX-2, interleukin-6 (IL-6), IL-1β, tumor necrosis factor-R (TNF-R), 5-lipoxygenase (5-LOX), hypoxia inducible factor-1R (HIF-1R), and vascular endothelial growth factor (VEGF), resulting in inflammation and tumorigenesis. Activation of NF-κB is induced by a cascade of events leading to the activation of inhibitor κB (IκB) kinases (IKKs), which in turn phosphorylates IκB. The subsequent ubiquitination and proteasomal degradation of IκB leaves NF-κB free to translocate to the nucleus. These kinases can be activated through phosphorylation by upstream kinases, including NFκB inducing kinase, mitogen-activated protein kinase (MAPK), and protein kinase C (PKC) (*44*). In addition, many studies have confirmed the cytokine function in the induction of transcription activity of NFκB through Janus kinase (JAK), extracellular signal-regulated protein kinase ½(Erk1/2)(p42/44), p38 MAPK, Ras, and phosphoinositide-3 kinase (PI3K)/Akt pathways (*45*). Therefore, agents that inhibit NF-κB pathway would decrease inflammatory response.

Black tea polyphenols have antioxidant activity, and many biological effects of black tea have been partially related to its antioxidant properties. However, anti-inflammatory properties of black tea have recently attracted more attention, particularly study of the cellular and molecular targets. Black tea extract has shown anti-inflammatory activity in the carrageenan-induced paw edema model in rats (*46*). The black tea constituent theaflavins were observed to inhibit TPA-induced edema of the mouse ear with an order of potency where TFDG > TF3G = TF3′G > TF (*47*). Many molecular targets that lead to inflammation have been shown to be affected by black tea. Administration of black tea extract equal to 40mg of black tea polyphenols/kg/rat inhibit azoxymethane (AOM)-induced expression of COX-2, iNOS, glutathione S-transferase (GST), GST-M2, and GST-P in colon tumors (*48*). Black tea polyphenols blocks the nitric oxide synthase by down-regulating the activation of NF-*k*B in activated murine macrophages (RAW 264.7 cell line), and TFDG is the most potent inhibitor of the activation of NF_B among black tea polyphenols (*49*). Application of TF and TFDG has been shown to inhibit 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced expression of IL-1 and IL-6 as well as increasing levels of PGE2 and LTB4 in mouse ear tissues (*47*). TF from black tea and TFDG in particular have been demonstrated to be effective anti-inflammatory agents. The beneficial anti-inflammatory effect was linked to theaflavins' ability to inhibit the activation of NF-κB by inhibiting the phosphorylation, subsequent degradation of IκBα, and suppression of expression of pro-inflammatory genes, including interferon-γ (IFN-γ), IL-12, TNF-α, and iNOS (*50*). TF also affects brain injury by blocking inflammation related events, including over-expression of COX-2 and iNOS via down-regulation of STAT-1 (signal transducers and activators of transcriptions) phosphorylation (*51*). Recently TF and TFDG were shown to modulate the regulators of G-protein signaling by selectively inducing the expression of regulator of G-protein signaling (RGS)-10 (*52*).

Downloaded by OHIO STATE UNIV LIBRARIES on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch004

Anti-Cancer

Various epidemiological studies have shown the correlation of tea consumption and cancer prevention, and the chemoprevention effects of tea have also been indicated in animal models at different sites including skin, lung, stomach, colon, pancreas, etc. (*53*). Besides anti-inflammation, inhibition of the cell proliferation and induction of apoptosis have been mostly evaluated as the major mechanism pathways for cancer prevention by black tea. The intracellular-signaling cascades, which have attracted more attention, could modulate gene expression to induce chemopreventive effects mentioned above. The MAPK family, which contains c-JUN N-terminal kinases/stress-activated protein kinases (JNKs/SAPKs), p38 kinases and the extracellular signal-regulated protein kinases (ERKs), has demonstrated important roles in the proliferation and apoptosis of tumor cells (*54*). When the MAPK pathways are activated, the transcription factors such as AP-1 and NF-κB binding to a specific DNA sequence induce the gene transcription. In addition, some Phase II enzymes, antioxidant enzymes can be also be activated by the transcription factor, nuclear factor-erythroid 2-related factor 2 (Nrf2) via MAPKs (*55*). Apoptosis, a form of programmed cell death, plays a critical role in both development and tissue homeostasis. The two main apoptotic pathways, the death receptor (extrinsic) and mitochondrial (intrinsic) pathway, are activated by caspase-8 and caspase-9, respectively (*56*). In the death receptor pathway, death receptors (Fas, TNFR etc.) interact with their ligands and recruit adaptor protein and initiator procaspases-8. Active initiator caspase-8 activates the effector caspase-3 to induce apoptosis or to initiate degradation of Bid and to yield cytochrome c release from mitochondria. The mitochondrial pathway involves release of cytochrome c and other mitochondrial molecules such as AIF and endo G, thereby forming the apoptosome which activates caspase-9 and is capable of proteolytically processing caspase-3 to induce apoptosis. The mitochondrial permeability also interacts with the Bcl-2 family of proteins. In the ER pathway, cytoplasmic Ca2+ concentration, as a result of ER stress, results in the activation of caspase-12, activation of JNK signaling and transcriptional induction of CHOP/GADD153. The phosphorylation of Bcl-2 has been described as an important step from microtubule damage to apoptosis.

The anti-cancer effect of theaflavins has been compared with EGCG in the ultraviolet (UV) B radiation induced- JB6 mouse epidermal cell line (*57*). The AP-1 activity is inhibited by the theaflavins in a concentration-dependent manner, and the effect of theaflavins is stronger than that of EGCG. In addition, theaflavins dramatically attenuate the activities of JNKs and ERKs. The structural effect of theaflavin (TF), theaflavin monogallates (TFG) and theaflavin digallate (TFDG) have been performed in SV-40 immortalited (33BES) and Ha-ras gene (21BES) transformed human bronchial epithelia cell lines (*58*). TFDG has shown the highest inhibitory effect of cell growth, and followed by TFG, suggesting the importance of gallate in the inhibition of cell growth. Meanwhile, the inhibition of c-JUN protein has been observed by the treatment with TFDG in 21BES cell lines. However, the inhibitory effect of various theaflavins on antiapoptotic Bcl-2 family has shown a contrary trend (*59*). No inhibition has been observed from TFDG, whereas theaflavanin shows the strongest ability to decrease the Bcl-2 expression. TF and thearubigins (TR) have demonstrated the induction of apoptosis through the mitochondrial pathway in the human skin cancer cells (*60*). In this process, BAX has been reported to translocate into mitochondria, release cytochrome c, and activate caspase-3. Meanwhile, up-regulation of p53 has also been observed. Recently, besides MAPKs, apoptosis caspases, apoptosis mediated by p53 has been shown as a new way to study cancer prevention. The primary function of the tumor suppressor p53, a sequence-specific transcription factor, can activate transcription of genes containing p53-binding sites, and induce the cell cycle arrest, apoptosis, and DNA repair. In mammary epithelial carcinoma cells, theaflavin induces the apoptosis via the p53-mediated Bax transactivation (*61*). It has been shown that apoptosis can be induced by theaflavin through Bax over expression even if p-53 expression is inhibited, whereas inhibition of Bax decreases the apoptotic effect.

Downloaded by OHIO STATE UNIV LIBRARIES on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch004

Conclusion

The chemistry and bio-functional properties of polyphenols in black tea have been discussed in this overview of their formation, taste properties, anti-inflammation and anti-cancer abilities. Theaflavins and thearubigins are the major polyphenols in black tea. The astringent perception of black tea is attributed to theaflavins and flavon-3-ol glycosides, and theaflavins have also demonstrated strong capabilities of anti-inflammation and anti-cancer via the NF-*k*B and MAPKs pathways, respectively.

References

1. Forrest, D. *The World Trade: A Survey of the Production, Distribution, and Consumption of Tea*; Woodhead-Faulkner, Ltd.: Cambridge, U.K., 1985.
2. Balentine, D. A. In *Phenolic Compounds in Food and Their Effects on Health I. Analysis, Occurrence, & Chemistry*; Ho, C. T., Lee, C. Y., Huang, M. T., Eds.; American Chemical Society: Washington, DC, 1998; pp 35−72.
3. Lin, Y. S.; Tsai, Y. J.; Tsai, J. S.; Lin, J. K. Factors affecting the levels of tea polyphenols and caffeine in tea leaves. *J. Agric. Food Chem.* **2003**, *51*, 1864–1873.
4. Nichenametla, S. N.; Taruscio, T. G.; Barney, D. L.; Exon, J. H. A review of the effects and mechanisms of polyphenolics in cancer. *Crit. Rev. Food Sci. Nutr.* **2006**, *46*, 161–183.
5. Klatsky, A. L.; Monton, C.; Udaltsova, N.; Friedman, D. Coffee, cirrhosis, and transaminase enzymes. *Arch. Intern. Med.* **2006**, *166*, 1190–1195.
6. Lakenbrink, C.; Lapczynski, S.; Maiwald, B.; Engelhardt, U. H. Flavonoids and other polyphenols in consumer brews of tea and other caffeinated beverages. *J Agric. Food. Chem.* **2000**, *48*, 2848–2852.

7. Lin, J. K.; Lin, C. L.; Liang, Y. C.; Lin-Shiau, S. Y.; Juan, I. M. Survey of catechins, gallic acid, and methylxanthines in green, oolong, pu'erh, and black teas. *J. Agric. Food Chem.* **1998**, *46*, 3635–3642.
8. Yamanishi, T.; Kawakami, M.; Kobayashi, A.; Hamada, T.; Musalam, Y. In *Thermal Generation of Aromas*; Parliment, T. H., McGorrin, R. J., Ho, C. T., Eds.; ACS Symp. Ser. 409; American Chemical Society: Washington, DC, 1992; pp 102−117.
9. Graham, H. N. In *The Methylxanthine Beverages and Foods: Chemistry, Consumption and Health Effects*; Spiller, G. A., Ed.; Alan R. Liss: New York, 1984; pp 29−74.
10. Roberts, E. A. H. Economic importance of flavonoid substances: Tea fermentation. In *The Chemistry of Flavonoid Compounds*; Geissman, T. A., Ed.; Pergamon Press: Oxford, U.K., 1962; pp 409−512.
11. Bradfield, A. E.; Penny, M. The catechins of green tea. Part Π. *J. Chem. Soc.* **1948**, 2249–2254.
12. Bradfield, A. E.; Batesmith, E. C. Chromatographic behaviour and chemical structure Π. The tea catechins. *Biochem. Biophys. Acta.* **1950**, *4*, 441–444.
13. Sanderson, G. W.; Ranadive, A. S.; Eisenberg, L. S.; Farrell, F. J.; Simons, R.; Manley, C. H.; Coggon, P. Contribution of polyphenolic compounds to the taste of tea. In *Phenolic, Sulfur, and Nitrogen Compounds in Food Flavors*; Charalambous, G., Katz, I., Eds.; ACS Symp. Ser.; American Chemical Society: Washington, DC, 1976; Vol. 26, pp 14−46.
14. Roberts, E. A. H.; Cartwright, R. A.; Oldschool, M. The phenolic substances of manufactured tea. I. Fractionation and paper chromatography of water-soluble substances. *J. Sci. Food Agric.* **1957**, *8*, 72–80.
15. Takino, Y.; Ferretti, A.; Flanagan, V.; Gianturco, M.; Vogel, M. Structure of theaflavin, a polyphenol of black tea. *Tetrahedron Lett.* **1965**, 4019–4025.
16. Bryce, T.; Collier, P. D.; Fowlis, I.; Thomas, P. E.; Frost, D.; Wilkins, C. K. The structures of the theaflavins of black tea. *Tetrahedron Lett.* **1970**, *11*, 2789–2792.
17. Collier, P. D.; Mallows, B. R.; Thomas, P. E.; Frost, D. J.; Korver, O.; Wilkins, C. K. The theaflavins of black tea. *Tetrahedron* **1973**, *29*, 125–142.
18. Coxon, D. T.; Holmes, A.; Ollis, W. D. Isotheaflavin. A new black tea pigment. *Tetrahedron Lett.* **1970**, *11*, 5241–5246.
19. Coxon, D. T.; Holmes, A.; Ollis, W. D.; Vora, V. C. The constitution and configuration of the theaflavin pigments of black tea. *Tetrahedron Lett.* **1970**, *11*, 5237–5240.
20. Robertson, A. In *Tea, Cultivation to Consumption*; Chapman & Hall: London, U.K., 1992; pp 555−601.
21. Wan, X.; Nursten, H. E.; Cai, Y.; Davis, A. L.; Wilkins, J. P. G.; Davis, A. P. A new type of tea pigment − From the chemical oxidation of epicatechin gallate and isolated from tea. *J. Sci. Food Agric.* **1997**, *74*, 401–408.
22. Lewis, J. R.; Davis, A. L.; Cai, Y.; Davies, A. P.; Wilkins, J. P. G.; Pennington, M. Theaflavate B, isotheaflavin-3′-*O*-gallate and neotheaflavin-3-*O*-gallate: Three polyphenolic pigments from black tea. *Phytochem.* **1998**, *49*, 2511–2519.

Downloaded by OHIO STATE UNIV LIBRARIES on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch004

23. Sang, S.; Tian, S. Y.; Meng, X. F.; Stark, R.; Rosen, R. T.; Yang, C. S.; Ho, C. T. Theadibenzotropolone A, a new type pigment from enzymatic oxidation of (−)-epicatechin and (–)-epigallocatechin gallate and characterized from black tea using LC/MS/MS. *Tetrahedron Lett.* **2002**, *43*, 7129–7133.
24. Brown, A. G.; Eyton, W. B.; Holmes, A.; Ollis, W. D. The identification of the thearubigins as polymeric proanthocyanidins. *Phytochem.* **1969**, *8*, 2333–2340.
25. Berkowitz, J. E.; Coggon, P.; Sanderson, G. W. Formation of epitheaflavic acid and its transformation to thearubigins during tea fermentation. *Phytochem.* **1971**, *10*, 2271–2278.
26. Obanda, M.; Owuro, P. O.; Mang'oka, R. Changes in the chemical and sensory quality parameters of black tea due to variations of fermentation time and temperature. *Food Chem.* **2001**, *75*, 395–404.
27. Robertson, A. *Phytochem.* **1983**, *22*, 889–896.
28. Scharbert, S.; Holzmann, N.; Hofmann, T. Identification of the astringent taste compounds in black tea infusions by combining instrumental analysis and human bioresponse. *J. Agric. Food Chem.* **2004**, *52*, 3498–3508.
29. Kaneko, S.; Kumazawa, K.; Matsuda, H.; Henze, A.; Hofmann, T. Molecular and sensory studies on the umami taste of Japanese green tea. *J. Agric. Food Chem.* **2006**, *54*, 2688–2694.
30. Ager, A. Inflammation: Border crossings. *Nature* **2003**, *421*, 703–705.
31. Stvrtinova, V.; Jakubovsky, J.; Hulin, I. *Pathophysiology Principles of Disease*; Academic Electronic Press: 1995; http://www.savba.sk/logos/books/scientific/Inffever.html.
32. Tipp, A. *Basic Pathophysiological Mechanisms of Inflammation*; McGraw-Hill Book Company: Lamoni, IA, 1979.
33. Finkel, T.; Holbrook, N. J. Oxidants, oxidative stress and the biology of ageing. *Nature* **2000**, *408*, 239–247.
34. Splettstoesser, W. D.; Schuff-Werner, P. Oxidative stress in phagocytes – The enemy within. *Microsc. Res. Tech.* **2002**, *57*, 441–455.
35. Finkel, T. Oxidant signals and oxidative stress. *Curr. Opin. Cell. Biol.* **2003**, *15*, 247–254.
36. Nelms, K. In *The Cytokine Network*; Balkwill, F., Ed.; Oxford University Press: London, U.K., 2000; pp 20−48
37. Brennan, F. M.; Feldmann, M. In *The Cytokine Network*; Balkwill, F., Ed.; Oxford University Press: London, U.K., 2000; pp 49−70.
38. Fernandez-Botran, R.; Vetvicka, V. *Methods in Cellular Immunology*; CRC Press: Boca Raton, FL, 2001.
39. Hussain, S. P.; Hofseth, L. J.; Harris, C. C. Radical causes of cancer. *Nature Rev. Cancer* **2003**, *3*, 276–285.
40. Ames, B. N.; Shigenaga, M. K.; Hagen, T. M. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 7915–7922.
41. Higg, G. A.; Eakins, K. E.; Moncada, S.; Vaqne, J. R. In *Prostaglandins and Inflammation*; Rainsford, K. D., Ford-Hutchson, A. W., Eds.; Basel: Birkhauser Verlag, 1999; pp 167−176.

Downloaded by OHIO STATE UNIV LIBRARIES on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch004

42. Balsinde, J.; Winstead, M. V.; Dennis, E. A. Phospholipase A2 regulation of arachidonic acid mobilization. *FEBS* **2002**, *531*, 2–6.
43. Bazan, N. G.; Colangelo, V.; Lukiw, W. J. Prostaglandins and other lipid mediators in Alzheimer's disease. *Prostaglandins Other Lipid Mediat.* **2002**, *68−69*, 197–210.
44. Maeda, S.; Omata, M. Inflammation and cancer: Role of nuclear factor-kappaB activation. *Cancer Sci.* **2008**, *99*, 836–842.
45. Agarwal, A.; Das, K.; Lerner, N.; Sathe, S.; Cicek, M.; Casey, G.; Sizemore, N. The AKT/IκB kinase pathway promotes angiogenic/metastatic gene expression in colorectal cancer by activating nuclear factor-κB and β-catenin. *Oncogene* **2005**, *24*, 1021–1031.
46. Liang, Y. C.; Tsai, T. C.; Lin-Shiau, S. Y.; Chen, C. F.; Ho, C. T.; Lin, J. K. Inhibition of 12-*O*-tetradecanoylphorbol-13-acetate-induced inflammatory skin edema and ornithine decarboxylase activity by theaflavin-3,3′-digallate in mouse. *Nutr. Cancer* **2002**, *42*, 217–223.
47. Huang, M. T.; Liu, Y.; Ramji, D.; Lo, C. Y.; Ghai, G.; Dunshenkov, S.; Ho, C. T. Inhibitory effects of black tea theaflavin derivatives on 12-O-tetradecanoylphorbol-13-acetate-induced inflammation and arachidonic acid metabolism in mouse ears. *Mol. Nutr. Food. Res.* **2006**, *50*, 115–122.
48. Luceri, C.; Cadermi, G.; Sanna, A.; Dolara, P. Tea polyphenols modulate the expression of cycloxygenase-2, inducible nitric oxide synthase and glutathione-related enzymes in azoxymethane-induced F344 rat colon tumors. *J. Nutr.* **2002**, *132*, 1376–1379.
49. Lin, Y. L.; Tsai, S. H.; Lin-Shiau, S. Y.; Ho, C. T.; Lin, J. K. Theaflavin-3,3'-digallate from black tea blocks the nitric oxide synthase by down-regulating the activation of NF-κB in macrophages. *Eur. J. Pharm.* **1999**, *19*, 379–388.
50. Ukil, A.; Maity, S.; Das, P. K. Protection from experimental colitis by theaflavin-3,3'-digallate correlates with inhibition of IKK and NF-κB activation. *Br. J. Pharmacol.* **2006**, *149*, 121–131.
51. Cai, F.; Li, C. R.; Wu, J. L.; Chen, J. G.; Liu, C.; Min, Q.; Yu, W.; Ouyang, C. H.; Chen, J. H. Theaflavin ameliorates cerebral ischemia-reperfusion injury in rats through its anti-inflammatory effect and modulation of STAT-1. *Mediators Inflamm.* **2006**, *5*, 1–9.
52. Lu, J.; Gosslau, A.; Liu, A. Y.; Chen, K. Y. PCR differential display-based identification of regulator of G protein signaling 10 as the target gene in human colon cancer cells induced by black tea polyphenol theaflavin monogallate. *Eur. J. Pharmacol.* **2008**, *601*, 66–72.
53. Shukla, Y. Tea and cancer chemoprevention: A comprehensive review. *Asian Pac. J. Cancer Prevention* **2007**, *8*, 155–166.
54. Kharbanda, S.; Saxena, S.; Yoshida, K.; Pandey, P.; Kaneki, M.; Wang, Q.; Cheng, K.; Chen, Y. N.; Campbell, A.; Sudha, T.; Yuan, Z. M.; Narula, J.; Weichselbaum, R.; Nalin, C.; Kufe, D. Translocation of SAPK/JNK to mitochondria and interaction with Bcl-x_L in response to DNA damage. *J. Biol. Chem.* **2000**, *275*, 322–327.
55. Surh, Y. J. Cancer chemoprevention with dietary phytochemicals. *Nat. Rev. Cancer* **2003**, *3*, 768.

Downloaded by OHIO STATE UNIV LIBRARIES on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch004

56. Pan, M. H.; Ho, C. T. Chemopreventive effects of natural dietary compounds on cancer development. *Chem. Soc. Rev.* **2008**, *37*, 2558–2574.
57. Nomura, M.; Ma, W. Y.; Huang, C.; Yang, C. S.; Bowden, G. T.; Miyamoto, K.; Dong, Z. Inhibition of ultraviolet B-induced AP-1 activation by theaflavins from black tea. *Mol. Carcinog.* **2000**, *28*, 148–155.
58. Yang, G. Y.; Liao, J.; Li, C.; Chung, J.; Yurkow, E. J.; Ho, C. T.; Yang, C. S. Effect of black tea and green tea polyphenols on c-Jun phosphorylation and H_2O_2 production in transformed and non-transformed human bronchial cell lines: Possible mechanisms of cell growth inhibition and apoptosis. *Carcinogenesis* **2000**, *21*, 2035–2039.
59. Leone, M.; Zhai, D.; Sareth, S.; Kitada, S.; Reed, J. C.; Pellecchia, M. Cancer prevention by tea polyphenols is linked to their direct inhibition of antiapoptotic Bcl-2-family proteins. *Cancer Res.* **2003**, *63*, 8118–8121.
60. Halder, B.; Bhattacharya, U.; Mukhopadhyay, S.; Giri, A. K. Molecular mechanism of black tea polyphenols induced apoptosis in human skin cancer cells: involvement of Bax translocation and mitochondria mediated death cascade. *Carcinogenesis* **2008**, *29*, 129–138.
61. Lahiry, L.; Saha, B.; Chakraborty, J.; Bhattacharyya, S.; Chattopadhyay, S.; Banerjee, S.; Choudhuri, T.; Mandal, D.; Bhattacharyya, A.; Sa, G.; Das, G. Contribution of p53-mediated Bax transactivation in theaflavin-induced mammary epithelial carcinoma cell apoptosis. *Apoptosis* **2008**, *13*, 771–781.

Downloaded by OHIO STATE UNIV LIBRARIES on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch004

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch005

Chapter 5

LC Taste® as a Tool for the Identification of Taste Modulating Compounds from Traditional African Teas

**K. V. Reichelt,[*,1] R. Peter,[2] M. Roloff,[2] J. P. Ley,[2] G. E. Krammer,[2]
K. M. Swanepoel,[3] and K.-H. Engel[1]**

**[1]Chair of General Food Technology, Technische Universität München, 85350
Freising – Weihenstephan, Germany
[2]Flavor & Nutrition, Research and Innovation, Symrise GmbH & Co. KG,
37603 Holzminden, Germany
[3]University of Zululand, KwaZulu Natal, South Africa
[*]Katharina.reichelt@symrise.com**

Taste modulating compounds are an important topic for the food industry. However, the identification of such compounds is difficult, time-consuming and laborious. To accelerate this process, a novel method was developed combining the separation of complex matrices by High Temperature Liquid Chromatography with sensory analysis. Based on this so-called LC Taste® approach, protocols for taste dilution analysis (TDA) and for the identification of taste modulating compounds were developed. Both methods were applied to extracts from Yerba Santa (*Eriodicyton angustifolium*) and two traditional African teas, honeybush tea (*Cyclopia intermedia*) and rooibos tea (*Aspalathus linearis*), to evaluate their taste modulating potential. Homoeriodictyol (**1**) and hesperetin (**3**) were identified as main taste modulating principles in Yerba Santa and honeybush, whereas no activity was detected for the supposed sweet compound, aspalathin (**7**) in rooibos tea.

In Flavors in Noncarbonated Beverages; Da Costa, N., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch005

Introduction

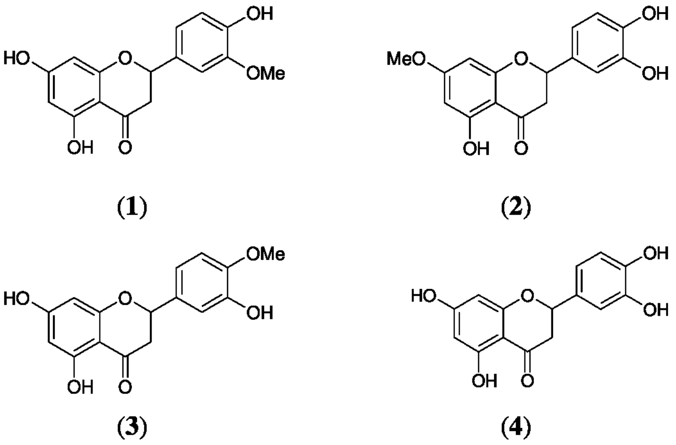
Taste and flavor modulating compounds, for example bitter maskers and sweet or salt enhancers, are currently an important topic in the food industry (*1–4*). As a reaction to the increasing prevalence of obesity and other life style-related diseases (*5*), consumers demand healthier foods and beverages. The reduction of sweet and caloric carbohydrates, like sucrose or high fructose corn syrup (HFCS), sodium chloride, or the fortification of foods with health-promoting ingredients confronts the food industry with a new challenge. Sugar reduction is often countered with the use of non-caloric, high-impact sweeteners. Yet these compounds often have the disadvantage of being artificial and showing different sweet profiles; bitter, metallic off-notes or lingering aftertaste compared to sugars. The use of sweet-enhancing compounds, which do not exhibit sweet taste themselves, but enhance the sweetness of sugars in a food preparation, might be an alternative to high-potency sweeteners. But not only artificial sweeteners can contribute to sensory deficiencies: foods enriched in health-promoting compounds, like polyphenols (e.g. catechins from green tea), soy products, vitamins or minerals tend to show unpleasant off-notes and change the taste profiles in a way that is not acceptable to the consumer.

Flavor modifiers are commonly defined as substances which have no typical flavoring properties *per se*, but are able to modify the flavor profile of other flavoring substances when used in combination (*6*).

In addition to the fact that these compounds have little or no intrinsic taste, but show their effects only in the presence of taste active compounds, detection of taste modifiers from natural sources is frequently hindered by the complexity of plant extracts this makes the work laborious as well as time- and cost- intensive. Well established methods used for the identification of taste active and flavor modifying compounds consist of simple duo-tests (*7*), as well as more sophisticated techniques such as comparative taste dilution analysis (cTDA) (*8*), which led to the identification of alapyraidine as a general taste enhancer. However, in general these methods are rather time-consuming, as the isolation of sufficient amounts of substances to carry out sensory analysis is required.

A wide range of LC methods has been established for the isolation of non-volatile compounds from complex natural products, e.g. preparative HPLC (pHPLC), or various liquid-liquid partition chromatography techniques, e.g. High Speed Countercurrent Chromatography (HSCCC) or Fast Centrifugal Partition Chromatography (FCPC) (*9*, *10*). However, all these techniques require the use of toxic solvents like methanol, acetonitrile, etc. As these solvents cannot be directly used for human taste evaluations, expensive and time-consuming steps for the isolation of single compounds or interesting fractions as well as removal of harmful solvents have to be performed prior to taste analysis to make the sensory analysis safe for the testers.

To avoid the use of harmful solvents, a novel method called LC Taste® (*12*) was developed, comparable to the well-established gas chromatography-olfactometry (GC-O), using the advantages of newly arising high temperature liquid chromatography (HTLC) (*11*). The use of higher temperatures changes the physicochemical properties of water and ethanol (*11*, *13–15*) and allows their



(1) **(2)** **(3)** **(4)**

*Figure 1. Selected Compounds from Eriodictyon sp.: homoeriodictyol (**1**), sterubin (**2**), hesperetin (**3**), eriodictyol (**4**).*

use as a solvent system for chromatographic separation of complex extracts, followed by coupled sensory analysis. This method allows data correlation from real-time analysis and *in vivo* detection of relevant taste active or taste modifying compounds, respectively, by a specially trained sensory panel.

Among the possible applications of LC Taste®, the identification of unpleasant off-notes or desirable taste active compounds in complex food matrices plays an important role. While conventional TDA is carried out by step-wise diluting a previously isolated compound, a method for coupled taste dilution analysis for LC Taste® was developed in order to establish a powerful tool for screening complex mixtures without previous isolation of compounds, modelled on the known combination of GC-O and AEDA. The principle of LC Taste® was performed on Yerba Santa extracts, a source of taste modulating compounds (*7*) as a test vehicle, as well as on different traditional African teas.

Two traditional African teas, representing different stages of economic development, were chosen as samples: rooibos tea (*Aspalathus linearis*) as well as honeybush tea (*Cyclopia intermedia*) (*16*). Fermented rooibos tea is one of the best known teas of South Africa and one of the few indigenous plants from South Africa that has gained commercial importance. It is free of caffeine and has a characteristic red-brown color and a pleasant sweetish flavor (*16*). For our experiments, unfermented rooibos tea was used, as it is described as containing the sweet principle aspalathin (**7**) (*17*), which is degraded during the fermentation process (*16*, *18*). Other compounds identified from rooibos tea are nothofagin (**8**), orientin, quercetin, as well as a number of different other flavonoids (*16*). Honeybush tea, produced from fermented *Cyclopia ssp.*, is traditionally used by the indigenous people of South Africa, but due to its pleasant honey-like flavor and its reported health benefits (low tannin content, caffeine free, high antioxidative capacity) it is becoming more and more popular in the western world (*16*). Among the identified compounds from *Cyclopia ssp.* are xanthones (mangiferin

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch005

(**6**), isomangiferin), flavanones and flavanone glycosides (e.g. hesperetin (**3**), hesperidin (**5**), naringenin), flavones, isoflavones and flavan-3-ols (*16*).

Experimental

Extraction and Fractionation of Plant Material

The dried material of *Eriodictyon angustifolium* (JPR Jenaer Pflanzenrohstoffe, Jena, Germany) was infused with boiling water. The steeped plant material was filtered, dried and extracted with methanol at room temperature, while stirring continuously. The extract was filtered and evaporated *in vacuo*.

Fermented and cut leaves of *Cyclopia intermedia* (honeybush tea, Alfred Galke GmbH, Gittelde/Harz, Germany) were extracted with methanol at room temperature while stirring continuously. The extract was filtered and evaporated *in vacuo*.

Unfermented aerial parts of *Aspalathus linearis* (rooibos tea, Adalbert Raps Zentrum für Arznei- & Gewürzpflanzenforschung, Freising/Weihenstephan, Germany) were extracted as described for honeybush tea.

LC Taste® fractionation of plant extracts for sensory evaluation was performed using High Temperature Liquid Chromatography on a polymer-based PRP-1 column in a semi-preparative scale (Hamilton, Bonaduz, Switzerland) using water/ethanol gradients at elevated temperature (120°C isotherm) and detection on a DAD detector (SunChrom SpectraFlow; wavelength range 200 - 400 nm, SunChrom, Friedrichsdorf, Germany). For taste modulation tests, pure water was fractionated under the same conditions as the corresponding extract and used as a reference for the corresponding fractions. Natural compounds were identified by re-analysis of the corresponding fractions by UV, HPLC-MS, and NMR analysis using standard compounds.

Sensory Evaluation

In principle, the taste of the individual fractions can be evaluated directly after elution from the column or time delayed, i.e. fractions are collected for the panellists to allow a comparison of fractions obtained from two different runs or samples. A successful on-line sensory evaluation via LC Taste® has recently been demonstrated using *Allium* extract as example (*19*). In this study the fractions were collected for practical reasons, and presented to panelists for further evaluation. For coupled taste dilution analysis, extracts were fractionated via LC Taste® either peak-wise or after defined time intervals. The fractions were presented to the testers, timed synchronously in decreasing order, asking them to describe the taste quality of the sample. The different dilution steps were achieved by 1+1 dilution of the stock solution with the appropriate solvent (i.e. ethanol, water, or mixtures of both). The procedure was repeated, separating the samples of the different

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch005

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch005

Table I. Sensory Evaluation of Yerba Santa Extract after Fractionation via LC Taste® and Compounds Contained in Selected Fractions

Fraction	*Taste quality*	*Compound*
1	Slightly bitter, astringent, slightly herbal	
2	Chamomile, medicinal, bitter	
3	Herbal, sweet, bitter (all weak)	
4	Tea-like, rum, phenolic, woody	
5	Slightly woody, sweeter	
6	Sweetish, weak	Eriodictyol (**4**)
7	Herbal, herbal, tea, slightly sweetish	Hesperetin (**3**)
8	Strong sweet, liquorices, strong bitter	Homoeriodictyol (**1**)
9	Slightly bitter, herbal, less sweet	
10	Slightly bitter, herbal, musty	Sterubin (**2**)
11	Bitter, unpleasant, medicinal, phenolic, smoky	
12	Bitter, smoky, chamomile	
13	Estery, fishy, cod liver	
14	Fishy, cod liver	
15	Bitter, fishy, blue mold cheese	
16	Fishy	
17	Fruity, sweet, artificial, camembert cheese, bitter, medicinal	
18	Herbal, bitter, type. Yerba Santa, musty, waxy	
19	Sweet, tea-like, liquorices (weak)	
20	Cod liver (weak), sweet	

concentration steps in decreasing order and sensorially evaluating them, until no taste was perceivable by the assessor. As in TDA, the last dilution factor, at which a taste could be perceived by the tester, is defined as the TD factor.

Tests for taste modulating compounds using LC Taste were carried out with the help of duo-tests, asking the panel which sample they perceived as more sweet or more bitter, respectively. The single fractions as well as a blank sample were blended with sucrose (5%) or caffeine solution (0.05%) in a ratio of 1:10. Because the concentration of each compound in a certain fraction may differ and the effect therefore cannot be based on the absolute activity of a single compound, a probability factor is introduced as a possibility to characterize the taste modulation effect of a particular fraction. The “taste modulation probability” (TMP) as an indicator for taste modulating compounds contained in a single fraction was calculated according to the following equation:

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch005

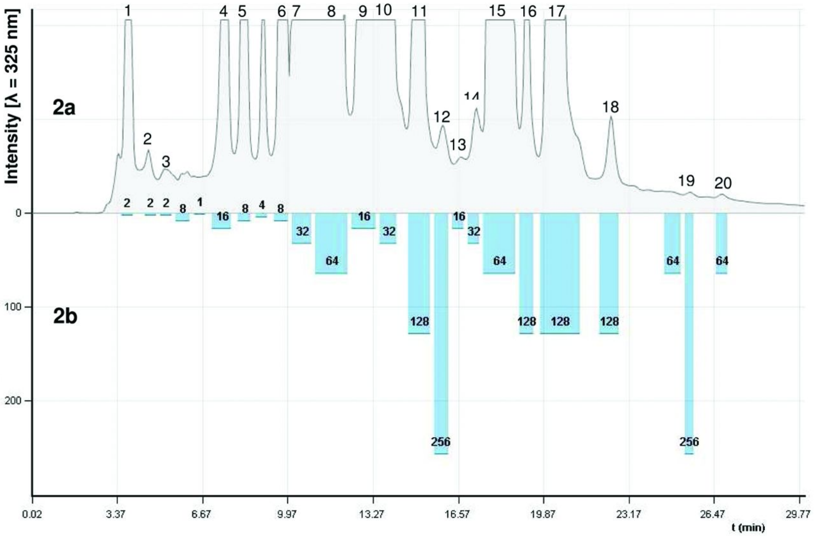


Figure 2. LC Taste® chromatogram of coupled sensory evaluation of Yerba Santa methanolic extract (Figure 2a, numbers indicate the fractions for sensory evaluation) and coupled Taste dilution analysis of Yerba Santa extract via LC Taste® (Figure 2b); effluent was evaluated in twenty fractions.

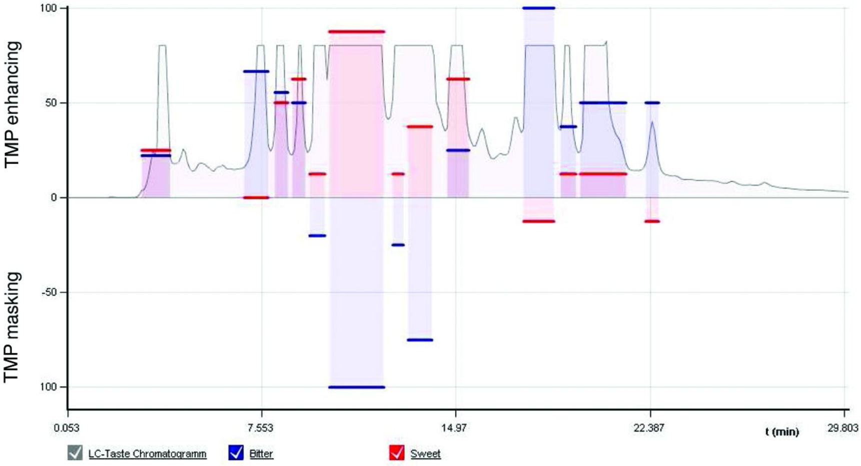


Figure 3. TMP values for Yerba Santa (E. angustifolium) methanolic extract determined in taste modulating trials on 5% sucrose (red) and 500 mg L^{-1} caffeine solution (blue) using LC Taste®. (see color insert)

$$\mathrm{TMP} = (\mathrm{n}_{\mathrm{higher}} - \mathrm{n}_{\mathrm{lower}}) / \mathrm{n}_{\mathrm{total}} \cdot 100 \qquad (1)$$

The TMP value describes the number of panelists experiencing a modulation effect of a fraction compared to chance. Therefore, the higher the TMP the higher the probability that there is a detectable effect. The TMP does not directly describe the maximum activity of single compound, but fractions with a high TMP show hints for strong modulators. Negative TMP values indicate reducing or masking effects, while positive TMP values stand for enhancing effects. Consequently, the TMP value reflects the probability that a compound or a fraction has taste modulating effects, but only gives indicative information about the intensity of the effect. To gain quantitative data, further tests have to be carried out, in which the panelists are asked to quote the intensity of the taste compared to the blank sample.

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch005

Sensory Evaluation of Teas via LC Taste®

Sensory Evaluation and Coupled Taste Dilution Analysis of an Extract from Yerba Santa Using LC Taste®

Yerba Santa extract is known for its taste modulating compounds homoeriodictyol (**1**) and hesperetin (**3**) (*7*) (Figure 1). Unfortunately, sensory evaluation of the crude extract did not reveal the same effect as the isolated substances (data not shown). To identify the reasons for this, Yerba Santa extract was chosen as model system for the development of new sensory evaluation protocols using the LC Taste® system.

Yerba Santa extract was fractionated peak-wise and evaluated sensorially by trained testers (Table I). To identify the key fractions contributing to the overall profile of the extract, coupled taste dilution analysis was carried out as described above.

Taste dilution analysis showed that especially the unpleasant and bitter tasting fractions, e.g. fractions 11, 12, 15 and 17, strongly contributed to the overall flavor of the Yerba Santa extract and made it difficult to detect the taste modulating effects of single compounds contained in the extract (Figure 2).

To circumvent this problem and to avoid time-consuming isolation steps, a new protocol for the identification of taste modulating compounds via LC Taste® was developed. Fractions were blended with caffeine or sucrose solution directly after elution from the LC Taste® system and tasted in a duo-test, compared to a blank sample. TMP values to estimate the masking or enhancing activity of a fraction were calculated as described in equation (1) and shown in Figure 3.

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch005

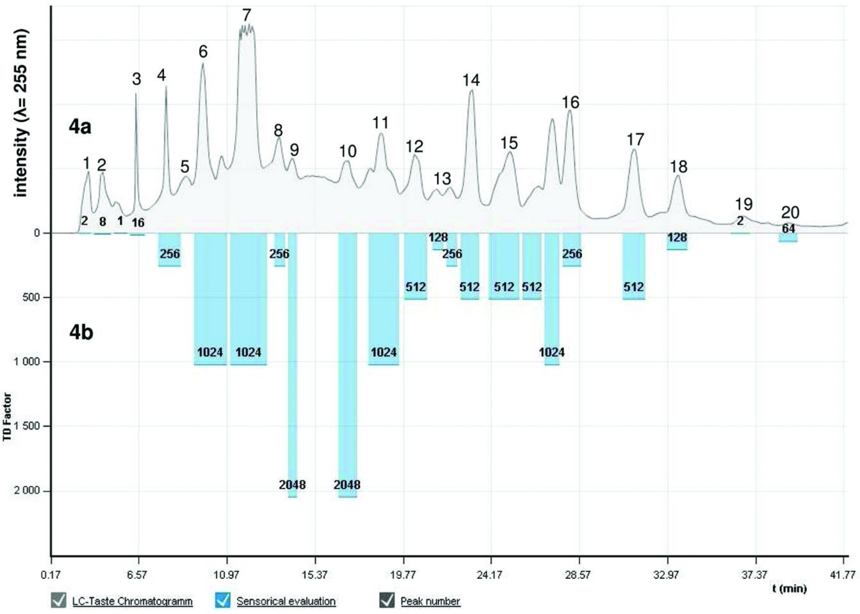


Figure 4. LC Taste® chromatogram of coupled sensory evaluation of honeybush methanolic extract (Figure 4a, numbers indicate the fractions for sensory evaluation) and coupled Taste dilution analysis of honeybush extract via LC Taste® (Figure 4b).

Sensory Evaluation and Coupled Taste Dilution Analysis of Honeybush Tea Using LC Taste®

Honeybush tea extract was fractionated via LC Taste® and sensorially evaluated peak-wise. The LC Taste® chromatogram is shown in Figure 4; results of the sensory evaluation are given in Table II.

Sensory evaluation of single peaks from honeybush extract showed a number of interesting taste impressions, for example sweet, honey-like or fruity. In addition, a number of typical, tea-like notes were described by the panellists, e.g. tea-like, bitter or herbal notes.

To find out which of these taste impressions contributed most to the overall flavor profile of honeybush tea, a taste dilution analysis via LC Taste® was carried out, as described above.

It was shown that especially fractions 9 and 10 (described as sweet and tea-like, but also woody) as well as fractions 6, 7 (tea-like, woody, sweet), 11 (medicinal, bitter) and 16 (very sweet, honey, liquorices-like), contributed strongly to the typical flavor profile of the tested honeybush extract.

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch005

Table II. Sensory evaluation of honeybush tea extract after fractionation via LC Taste® and compounds contained in selected fractions

Fraction	*Taste quality*	*Compound*
1	Bitter, musty, dry, tea, metallic (weak)	
2	Bitter (weak), fruity	
3	Bitter (weak), musty, medicinal	
4	Bitter, musty, clove	
5	Fruity, tea-like, musty, mouth-drying	
6	Weak, tea-like, musty, woody	
7	Woody, astringent, sweet, phenolic, burned, chamomile, astringent	Mangiferin (**6**)
8	Bitter, woody (weak), sweetish, raspberry	
9	Woody, phenolic, clove, tea-like, fruity (weak), sweet	
10	Sweet, tea-like, woody (weak), medicinal (weak)	
11	Raspberry, medicinal, bitter	Hesperidin (**5**)
12	Raspberry, sweet, woody (weak), rum	
13	Bitter, medicinal, fruity, tea-like	
14	Fruity, phenolic, fishy (weak), hay	
15	Animalic, phenolic, sweet, cerealic, caraway, coriander	
16	Very sweet, honey, liquorice	Hesperetin (**3**)
17	Fishy (weak), bitter (chicory-type), sweet, musty	
18	Bitter, green, kohlrabi, unpleasant	
19	Sweetish, fruity (weak), bitterer	
20	Bitter, honey, raspberry	

Identification of Taste Modulating Compounds from Honeybush Tea Extract Using LC Taste®

To identify taste modulating compounds in honeybush tea, single fractions were blended with caffeine and sucrose solution, as described for Yerba Santa, and evaluated by a trained panel using duo-tests to determine the TMP values. As shown in Figure 5, fractions 11 and 16 showed increased TMP values in terms of sweet enhancing. LC-MS analysis of these two fractions revealed that they contained the flavonoid glycoside hesperidin (**5**) and its aglycon hesperetin (**3**), respectively (Figure 6). The latter one already showed sweet enhancing effects in the LC Taste® taste modulating tests with Yerba Santa extract. Results were

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch005

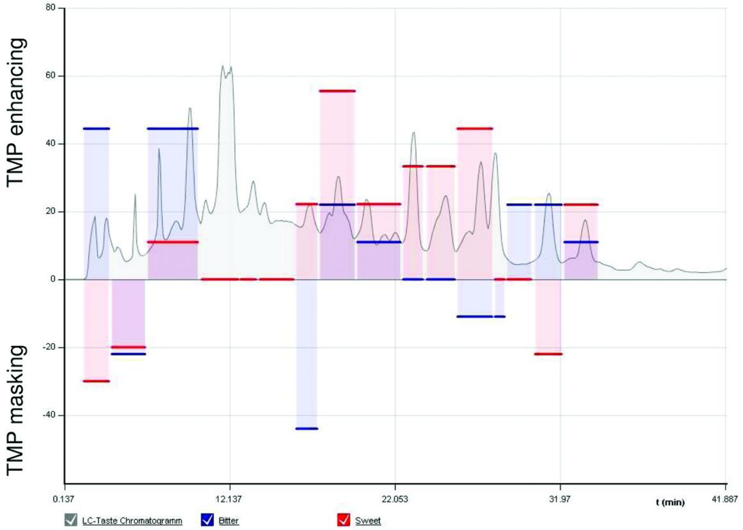
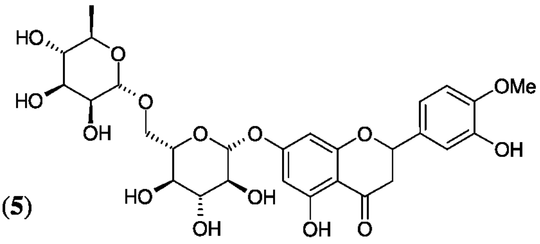
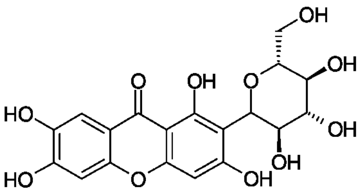


Figure 5. TMP values for honeybush (C. intermedia) methanolic extract determined in taste modulating trials on 5% sucrose (red) and 500 mg L^{-1} caffeine solution (blue) using LC Taste®. (see color insert)



(5)

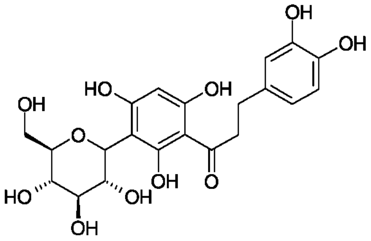


(6)

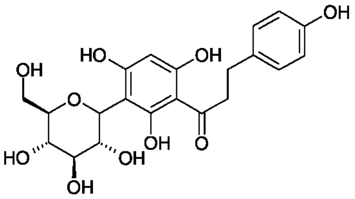
*Figure 6. Selected Compounds from Cyclopia intermedia: hesperidin (**5**) and mangiferin (**6**).*

confirmed with pure hesperetin using a conventional duo test in comparison to 5% sucrose solution.

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch005



(7)



(8)

Figure 7. Selected Compounds from Aspalathus linearis: aspalathin (7) and nothofagin (8).

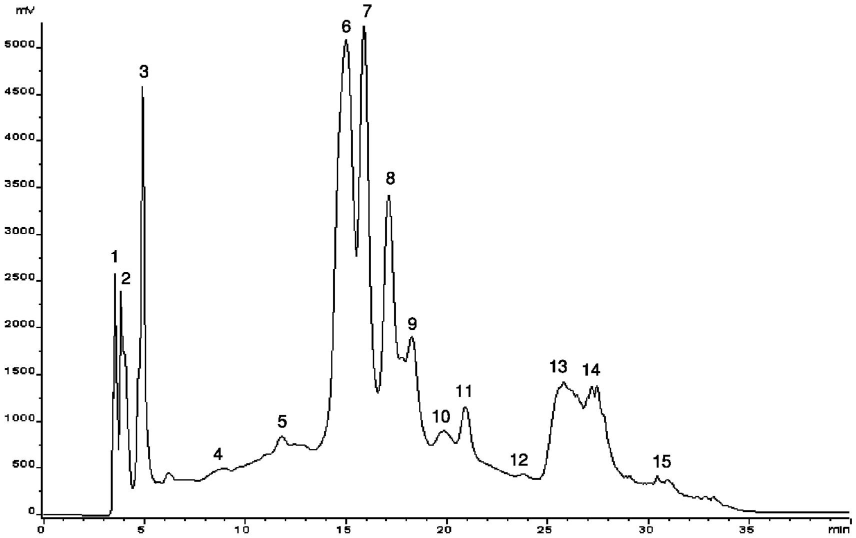


Figure 8. LC Taste® chromatogram of unfermented rooibos tea extract (numbers refer to the fractions for sensory evaluation).

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch005

Table III. Sensory evaluation of rooibos tea extract after fractionation via LC Taste®

Fraction	*Taste quality*	*Compound*
1	Sweet (weak)	
2	Herbal, tea (weak)	
3	Bitter, plastic	
4	Bitter, herbal, woody, musty	
5	Fruity, herbal, tea-like	
6	Herbal, musty, bitter, sweetish (weak)	Aspalathin (**7**)
7	More bitter	
8	Bitter	
9	Bitter	
10	Bitter	
11	Bitter, tea-like	Nothofagin (**8**)
12	Bitter, sweeter, tea-like	
13	Bitter, earthy, woody	
14	Fruity, sweet, bitter, honey, raspberry	
15	Sweetish, bitter, honey, tea-like	
16	Green, bitter, hay-like	

Sensory Evaluation of Non-Fermented Rooibos Tea Extract Using LC Taste®

In addition to honeybush tea extract, an extract of unfermented rooibos tea was fractionated via LC Taste® (Figure 8) and evaluated sensorially, to detect the sweet taste of aspalathin (**7**) (Figure 7), which has been described in the literature (*17*). Fractions were analyzed by LC-MS to identify the compounds contained in the single fractions. In addition, the identity of aspalathin was confirmed using isolated aspalathin (**7**) as a reference.

Sensory evaluation by trained assessors showed a number of fractions having bitter, tea-like and herbal notes, but also a few fractions exhibiting a sweetish, honey-like taste (Table III). Fraction 6, however, containing aspalathin (**7**), showed herbal, musty and bitter notes, but only a weak sweetish taste. To confirm the results of the sensory evaluation by LC Taste®, pure aspalathin was evaluated sensorially at a concentration of 100ppm in water, as well as in combination with sucrose (5%) and caffeine (500ppm) solutions, to detect possible taste modulating effects. These tests showed neither sweet nor sweet enhancing nor bitter masking effects for aspalathin (**7**), so that the literature data (*17*) could not be confirmed in these experiments.

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch005

Conclusion

LC Taste ® is a powerful and fast screening tool for taste and flavor active compounds in a complex mixture such as raw or pre-fractionated plant extracts. Besides the simple taste screening, more advanced experiments such as a modified TDA and taste modulation effects can be performed in a reasonable time without complex evaporation and re-dilution steps. It is not intended for use as an approach to replace classical fractionation, characterization and thorough evaluation of promising single compounds, but it will help to sort out the low- or non-active compounds in complex plant extracts.

References

1. Toldra, F.; Barat, J. M. Recent Patents for Sodium Reduction in Foods. *Recent Patents Food, Nutr., Agric.* **2009**, *1* (1), 80–86.
2. Winkel, C.; de Klerk, A.; Visser, J.; Bakker, J.; Koenig, T.; Rennes, H. New Developments in Umami (Enhancing) Molecules. *Chem. Biodiversity* **2008**, *5* (6), 1195–1203.
3. DuBois, G. E. *Sweeteners and Sweetness Modulators: Requirements for Commercial Viability*; Oxford University Press: New York, 2008.
4. Ley, J. P. Masking Bitter Taste by Molecules. *Chemosen. Percep.* **2008**, *1* (1), 58–77.
5. Schulze, M. B.; Manson, J. E.; Ludwig, D. S.; Colditz, G. A.; Stampfer, M. J.; Willett, W. C.; Hu, F. B. Sugar Sweetened Beverages, Weight Gain, and Incidence of Type 2 Diabetes in Young and Middle-Aged Women. *JAMA* **2004**, *292* (8), 927–934.
6. Krammer, G. E. Personal communication.
7. Ley, J. P.; Krammer, G. E.; Reinders, G.; Gatfield, I. L.; Bertram, H.-J. Evaluation of Bitter Masking Flavanones from Herba Santa (*Eriodictyon californicum* (H. & A.) Torr., Hydrophyllaceae). *J. Agric. Food Chem.* **2005**, *53* (15), 6061–6066.
8. Soldo, T.; Hofmann, T. Application of Hydrophyllic Interaction Liquid Chromatography/Comparative Taste Dilution Analysis for Identification of a Bitter Inhibitor by a Combinatorial Approach Based on Maillard Reaction Chemistry. *J. Agric. Food Chem.* **2005**, *53* (23), 9165–9171.
9. McAlpine, J. B.; Morris, P. Separation by High-Speed Countercurrent Chromatography. In *Natural Products Isolation*, 2nd ed.; Sarker, S. D., Latif, Z., Gray, A. I., Eds.; Humana Press: Totowa, NJ, 2006; pp 185−212.
10. Latif, Z. Isolation by Preparative High-Performance Liquid Chromatography. In *Natural Products Isolation*, 2nd ed.; Sarker, S. D., Latif, Z., Gray, A. I., Eds.; Humana Press: Totowa, NJ, 2006; pp 213−232.
11. Yang, Y. Subcritical Water Chromatography: A Green Approach to High-Temperature Liquid Chromatography. *J. Sep. Sci.* **2007**, *30*, 1131–1140.

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch005

12. Roloff, M.; Erfurt, H.; Kindel, G.; Schmidt, C. O.; Krammer, G. Process for the Separation and Sensory Evaluation of Flavours. Patent WO2006/111476, 2006.
13. Khuwijitjaru, P.; Adachi, S.; Matsuno, R. Solubility of Saturated Fatty Acids in Water at Elevated Temperatures. *Biosci. Biotech. Biochem.* **2002**, *66* (8), 1723–1726.
14. Smith, R. M. Extractions with Superheated Water. *J. Chromatogr., A* **2002**, *975*, 31–46.
15. Miller, D. J.; Hawthorne, S. B. Method for Determining the Solubilities of Hydrophobic Organics in Subcritical Water. *Anal. Chem.* **1998**, *70* (8), 1618–1621.
16. Joubert, E.; Gelderblom, W. C. A.; Louw, A.; de Beer, D. South African herbal teas: *Aspalathus linearis, Cyclopia* ssp. and *Athrixia phylicoides* – A review. *J. Ethnopharm.* **2008**, *119*, 376–412.
17. Bohm, B. A. Human Uses of Flavonoids. In *Introduction to Flavonoids (Chemistry and Biochemistry of Organic Natural Products)*; Harwood Academic Publishers: Newark, NJ, 1998; Vol. 2, pp 365−393.
18. Krafczyk, N.; Glomb, M. A. Characterization of Phenolic Compounds in Rooibos Tea. *J. Agric. Food Chem.* **2008**, *56* (9), 3368–3376.
19. (a) Krammer, G.; Sabater, C.; Brennecke, S.; Liebig, M.; Freiherr, K.; Ott, F.; Ley, J. P.; Weber, B.; Stöckigt, D.; Roloff, M.; Schmidt, C. O.; Gatfield, I.; Bertram, H. J. The Flavor Chemistry of Culinary Allium Preparations, Weurman Flavour Research Symposium, Roskilde , 2005. (b) Krammer, G.; Sabater, C.; Brennecke, S.; Liebig, M.; Freiherr, K.; Ott, F.; Ley, J. P.; Weber, B.; Stöckigt, D.; Roloff, M.; Schmidt, C. O.; Gatfield, I.; Bertram, H. J. The Flavor Chemistry of Culinary Allium Preparations. In *Flavor Science – Recent Advances and Trends*; Bredie, W. L. P., Petersen, M. A., Eds.; Elsevier: Amsterdam, The Netherlands, 2006; pp 169−172.

Downloaded by UNIV OF GUELPH LIBRARY on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch006

Chapter 6

The Key Odorants of Coffee from Various Geographical Locations

Robert J. Cannon,*[*] Laurence Trinnaman, Brian Grainger, and Amy Trail

International Flavors & Fragrances Inc., 1515 Highway 36, Union Beach, NJ 07735, USA
[*]robert.cannon@iff.com

The unique and complex taste of coffee has drawn great attention in the flavor industry over the past few decades (*1*, *2*). Five geographical varietals of Arabica beans (Colombia, Costa Rica, Java, Kenya and Suluwesi), as well as a Robusta bean and an Instant brand, were selected to understand the differences between the flavor profiles of the various medium roast beans. Sensory and analytical techniques were used to study the brewed coffees, with the ultimate goal being to link the two techniques. Using SPME (static headspace), Twister™ (stir bar sorptive extraction) and liquid-liquid extraction, each brewed coffee varietal was analyzed and profiled. Gas Chromatography-Mass Spectrometry (GC-MS) revealed over six hundred volatile and semi-volatile components. In addition, an expert panel evaluated and characterized the varieties of coffees, using comparison charts to understand the sensory profiles. From these two in-depth studies, work was done to link analytical and sensory, and thus help understand which chemical drivers create the taste differences.

Introduction

Coffee drinkers from all over the world are drawn to certain flavor profiles. There are many factors that create flavor differences among coffee varietals, including the geographic origin of the bean and the temperature at which the bean

In Flavors in Noncarbonated Beverages; Da Costa, N., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

was roasted. Attempts by research teams to study the flavor differences for coffee using analytical techniques have proved to be quite difficult but there has been some progress. Previous work done by Nestlé showed a standardized mechanism to predict sensory profiles using an on-line analytical measurement technique from the headspace of the coffee (*3*). A different approach to the link between composition and sensory perception was taken in this research with the ultimate goal of providing our flavorists with an understanding of what consumers are drawn to in certain flavor profiles of coffee beans. Therefore, this research sought to correlate the analytical and sensory studies for the different geographical beans.

The correlations fell into several broad categories. First, comparisons were made for the five Medium Roast Arabica beans as well as the Medium Roast Robusta bean, specifically studying the winey/berry notes and the pyrazine notes. This study was done by stir bar sorptive extraction (Twister™). Secondly, quantification and comparisons were made from the chromatographic analyses of the liquid-liquid extracts for the sugar degradation products between an Arabica bean and a Robusta bean. Finally, this research led to interesting discoveries between the three roasting temperatures; Low Roast (215°C), Medium Roast (230°C), and High Roast (245°C), of a particular Arabica bean. These conclusions were made from measurements of trigonelline through HPLC-UV and pyridine derivatives by gas chromatography of the liquid-liquid extract. Using both analytical and sensory measurements, research was done to identify the main compositional differences between various geographical coffee beans, as well as discover the key chemical drivers that create these flavor differences.

In-depth analytical research led to the identification of several hundred compounds from each of the analytical extraction techniques. As GC-O, stable isotope dilution assays (SIDA), and flavor dilution factors have shown, only 25-40 organoleptically important compounds contribute to the coffee aroma and flavor (*2*, *4*). Therefore, it would not be practicable to make comparisons and conclusions for sensory from such a large study. Thus we chose to monitor just the following compounds (Table I) as determined by an in-house sensory panel.

In order to support the analytical effort, a trained sensory panel produced and used spider charts to profile, evaluate and compare the coffee varietals (Figure 1). The descriptors around the chart were chosen by the panelists to relate the flavor impression given during the tasting panel. A rating from zero to ten was set for each descriptor, with ten being the highest a particular note would be found in any type of coffee.

Interesting discoveries and trends were established when linking analytical and sensory measurements. The learning's from the sensory paneling helped to narrow the scope of the analytical work for the volatile components, specifically the pyrazine notes and the sugar degradation compounds. There were also links made between the roasting temperature of the coffee beans and the flavor profile. Finally, some research was done to begin to link the semi- and non-volatile compounds with the taste of the coffee, specifically the organic acids and bitter compounds.

Downloaded by UNIV OF GUELPH LIBRARY on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch006

Downloaded by UNIV OF GUELPH LIBRARY on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch006

Materials and Methods

Five Gold Standard Arabica Coffee Beans were chosen for analysis (Kenyan, Sulawesi, Colombia, Costa Rica and Java), along with one Indonesian Robusta Bean and one Instant Coffee (Nescafe® Taster's Choice®). The origins of the beans were chosen to give a wide range of sensory character, yet using ones which were common items of commerce, rather than exotics. The five Arabica bean types and the Indonesian Robusta bean were purchased from Kaffé Magnum Opus® Company. All six coffee bean types were roasted to three different degrees; 215°C-light roast, 230°C-medium roast and 245°C-dark roast respectively, resulting in nineteen samples in total. Distilled water and a French Press Pot were used to brew the coffee beans. Dichloromethane (99.9%, Acros) was used as the liquid extraction solvent.

Liquid-Liquid Extraction

A beaker containing 800g of distilled water was microwaved for four minutes and forty-five seconds to allow the water to reach approximately 87°C. A measured amount of 50g of coffee beans was added to a coffee grinder. The beans were ground for twenty seconds and added to a French Press coffee brewer. The microwaved water was added to the ground beans and stirred for five seconds. The mixture was allowed to brew for three minutes. After filtering, the brewed coffee was added to an Erlenmeyer flask with 300mL of distilled cold water. This flask was submerged immediately in an ice bath to bring the temperature of the coffee down as quickly as possible. This was done twice and added together to be extracted with 600mL of methylene chloride (2 x 300mL), dried over anhydrous sodium sulfate, filtered, and concentrated by rotary evaporation to 1mL. The extract was analyzed by Gas Chromatography, Gas Chromatography-Mass Spectrometry, and Gas-Chromatography Olfactometry promptly after extraction to limit degradation.

Stir Bar Sorptive Extraction (Twister™)

The brewing and cooling procedure was followed according to the above protocol. The solution was added to Twister™ (Gerstel Inc., Baltimore, MD) vessels and sampled for one hour and two hours using the standard PDMS coated Twister™ on a setting of 1500rpm for the stirrer plate. The Twister™ samples were thermally desorbed onto a HP6890 (Hewlett Packard, Wilmington, PA) gas chromatograph equipped with a flame ionization detector (FID) using a Gerstel thermal desorber Model TDS 2. Desorption time was 5 min at 250°C. The column was an OV-1 capillary column and the analysis was performed in splitless mode. The injector temperature was programmed from 150°C (held for 5 min during the thermal desorption) to 250°C. Detector temperature was 320°C.

Downloaded by UNIV OF GUELPH LIBRARY on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch006

Gas Chromatography

The samples were analyzed using a HP6890 GC with 50m OV-1 capillary column (50m x 0.32mm i.d., 0.5μm film thickness) with split ratio of 40:1 or in the splitless mode, temperature program 40°C to 270°C @ 2°C/min with a hold at 270°C of 10mins. For GC-Olfactometry sessions, extract was injected onto a HP5890 GC with odor port Gerstel ODP-2. The GC conditions were the same as described above. All data was collected and stored by using HP ChemStation software.

Gas Chromatography-Mass Spectrometry

The identification of components was conducted by mass spectrometry. The samples were injected onto an HP6890 GC. The chromatographic conditions for the OV-1 column were the same as described for GC analysis. The end of the GC capillary column was inserted directly into the ion source of the mass spectrometer via a heated transfer line maintained at 280°C. The mass spectrometer was a Micromass Prospec high resolution, double-focusing, magnetic sector instrument. The mass spectrometer was operated in the electron ionization mode (EI), scanning from *m/z* 450 to *m/z* 33 at 0.3 seconds per decade.

Polar GC-MS analysis was conducted on a Carbowax capillary column (50m x 0.32mm i.d., 0.3μm film thickness); the sample was introduced via an HP5890 GC into a Kratos Profile mass spectrometer (Manchester, UK). The temperature program began with an initial temperature of 60°C held for 10 mins, ramped at 2°C/min to a final temperature of 220°C and held for 20 mins. The mass spectrometer was operated in EI mode scanning from *m/z* 450 to *m/z* 33 @ 0.3 sec per decade.

Spectra obtained from both phases were interpreted on a MassLib data system (Max Planck Institute, Germany), using IFF in-house libraries and commercial Wiley 8, NIST 98 and other libraries. The identification of components was confirmed by interpretation of MS data and by relative GC retention indices based on a calibration with ethyl esters.

High Performance Liquid Chromatography (HPLC)

Each coffee varietal was brewed the same way as the Liquid-Liquid extraction protocol. An aliquot of sample was filtered with 0.45μm nylon filter and submitted for HPLC analysis. Trigonelline was measured on a HPLC-UV Agilent Technologies 1100LC: Tosoh Amide-80 Column, UV @ 276nm detection.

Sensory Evaluation

The coffee beans were ground with a Burr Grinder and approximately 50g of the ground coffee was added to 800mL of water (~87-90°C) in a French Press. The solution was stirred for five seconds and allowed to brew for three minutes.

Downloaded by UNIV OF GUELPH LIBRARY on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch006

Taster's Choice Instant coffee was prepared to recipe, which was 1.3g of coffee per 100mL of hot water (~87-90°C).

A technical flavor panel composed of sensory, beverage applications, and flavorists evaluated the brewed coffee samples. A total of eight trained and experienced members were involved. The panel evaluated aroma, flavor, and aftertaste of each sample in open cups. The attributes included in the spider charts were agreed on previous to the tasting. The panelists evaluated the aftertaste one minute after swallowing. The spider charts for this paper were created for the flavor during the drinking of the coffee. The panelists used a scale from one to ten and were trained with certain reference compounds as anchors. Aroma profiles were also made from the sniffing prior to drinking, but these results have not bee presented. A maximum of three samples were evaluated in one session. Samples were evaluated within five minutes of brewing. Consensus profiles were created from individual scoring to create profile plots.

Results and Discussion

One of the dominant aroma and flavor impressions in coffee that helps differentiate it so well from other beverages comes from the pyrazine compounds. These heterocyclic compounds are formed by a Maillard reaction and Strecker degradation between reducing sugars and amino acids (*5*, *6*). They are known to give nutty, earthy, dirty notes in coffee.

Most literature suggests that 2-methylisoborneol, geosmin, and 2,4,6-trichloroanisole (Figure 2) are found at higher concentrations in Robusta bean compared to Arabica beans and, also contribute to the dirty, earthy, and musty notes of the flavor (*7*). An opposing article by Blank and Grosch (*8*) stated that 2-methylisoborneol does not play as big a role in coffee as most research suggests. Sensory panels do in fact show that there are differences in the earthy-type notes for Arabica and Robusta beans. Analytical results on these compounds were inconclusive and could not link up with what was found with sensory.

However, our results show that the pyrazine compounds also aided in this earthy impression as well as giving notes of nutty, cereal/toasted grain, and cocoa to the brewed coffee. Throughout this research, it was evident that both sensory and analytical found differences with the pyrazine notes among the coffee varietals. When the flavor was dominated by the pyrazine notes, the sensory panel revealed the overall impression of the brewed coffee to be a negative to the profile. Analytically, this is certainly evident in the Robusta bean where there were very high levels of pyrazine compounds compared to the Arabica beans.

According to the sensory panels, a major difference among the coffee varietals was the "pyrazine" notes (Figure 3). The sensory panels used notes of cocoa, cereal/toasted grain, nutty, and earthy to describe many of the pyrazine compounds found in coffee.

The different Arabica beans gave a wide range of results for the pyrazine notes. For example, the Medium Roast Robusta brew gave more of a cereal/toasted grain and earthy impression. Analytically, there were comparatively higher

In Flavors in Noncarbonated Beverages; Da Costa, N., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

Table I. Key Odorants of Coffee with Corresponding Odor and/or Flavor

#	*Component Name*	*Odor/Flavor*
1	2-Ethyl-3,5-dimethylpyrazine	Burnt almond, woody, nutty, roasted, earthy
2	2-Ethyl-3,6-dimethylpyrazine	Hazelnut, earthy, baked, roasted
3	2-isoButyl-3-methoxypyrazine	Green, bell-pepper, peas, slightly earthy
4	2-Methoxy-4-vinylphenol	Smoky, sweet, spicy
5	2-Methylbutanal	Chocolate-like
6	4-Ethylguaiacol	Smoky, roasted, burnt
7	5-Methylcyclopentapyrazine	Peanut, earthy, baked, potato-like
8	Cyclotene	Caramel, maple, woody
9	Difurfuryl sulfide	Onion, garlic, meaty, mushroom, caramel
10	Dimethyl disulfide	Intensely onion-like
11	Dimethyl trisulfide	Cabbage, burnt, cooked
12	Furaneol	Strawberry, sweet
13	Furfuryl mercaptan	Coffee-like, caramellic-burnt, sweet
14	Furfuryl methyl disulfide	Alliaceous, burnt
15	Furfuryl methyl sulfide	Strong, mustard, garlic, burnt
16	Guaiacol	Smoky, woody, phenolic, meaty
17	isoValeraldehyde	Malt, chocolate
18	Linalool	Floral, green, woody
19	Maltol	Fruity, caramellic, berry
20	Methional	Potato-like, tomato
21	Pentan-2,3-dione	Oily, buttery
22	Phenylacetaldehyde	Green, floral, sweet, honey
23	Propionic acid	Sour, pleasant, cheesy-sour
24	(*E*)-2-Nonenal	Fresh-brewed, woody, fatty, cucumber
25	(*E*)-*β*-Damascenone	Fruity, red-fruity, woody, sweet
26	Trimethylpyrazine	Nutty, roasted, coffee-like

concentrations of 2-ethyl-3,5(6)-dimethylpyrazine and trimethylpyrazine in this sample, which correlated to the particular notes perceived by the panelists. The Costa Rican bean gave a higher nutty impression to the brewed coffee and 5-methyl-6,7-dihydro-5H-cylcopentapyrazine was a potent peanut-like note that was found at higher concentrations compared to the rest of the coffee varietals.

Another trend discovered from the sensory paneling came from the winey/berry notes of the brewed coffees. Each varietal showed major differences between each other (Figure 4). The winey/berry notes were described by the panelists as

Downloaded by UNIV OF GUELPH LIBRARY on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch006

giving a catty, green/vegetative, green, raisin/prune, winey/fermented fruit, berry/plum, and citrus-like impression to the brewed coffee.

There were some important links made for the Sulawesi brew. As Figure 4 shows, this coffee only gave one strong green note, which the panelists used to describe floral and herbal notes. When looked at comparatively the potent "winey/berry" odorants in the analytical results, the linalool concentration was approximately 4-6 stronger in area counts compared to the other coffee varietals. Linalool can be described as woody, floral, and green in odor and flavor.

The Costa Rican brewed coffee gave the panelists a green, vegetative impression. A well-know potent odorant in green coffee is 2-isobutyl-3-methoxypyrazine (*1*). This compound is found at high concentrations in the green bean. During the roasting process, this compound degrades and is therefore found at much lower concentrations. It gives a green bell-pepper note and was found at relatively higher concentrations in the Costa Rican brew compared to the other coffees.

One of the major impressions that the sensory panel had for the Java brew was high catty notes. These catty notes can be associated with a majority of the potent sulfur molecules found in coffee, including difurfuryl sulfide, dimethyl disulfide, dimethyl trisulfide, furfuryl methyl disulfide, and furfuryl methyl sulfide. Studies were done on all of these sulfur compounds for each of the coffee varietals. The area count levels were significantly higher for the Java brew compared to the other coffees. This was an important link made between sensory and analytical for understanding the elevated catty notes in the Java flavor.

It is important to acknowledge the potential contradiction presented by other research teams that reported difficulty in identifying single compound aromas within a mixture. Individual compound aromas in a mixture can be suppressed, masked or create a new aroma, which seems to depend on the chemical polarity and ability to diffuse through olfactory mucus and ultimately to the receptor neurons to activate a signal to the brain. There have also been studies that discuss the limited ability of humans to identify certain components in a complex mixture (*9–12*). As mentioned earlier, although brewed coffee is a complex matrix of flavor compounds, only a handful of compounds contribute to the overall flavor. The sensory panelling done through our research used spider charts that established aroma and flavor notes known to brewed coffee. Reference compounds were used as anchors for the panellists to correlate the notes that were perceived.

The second discovery made from this research came when analyzing the sugar degradation products of the Arabica and Robusta beans. When the panel made their sensory measurements for the Medium Roast Kenyan (Arabica) bean and the Medium Roast Robusta bean (Figure 5), there seemed to be a very dominant impression for the sugar degradation notes with the Kenya flavor compared to the Robusta flavor.

The analytical results matched the sensory findings. About 8-10 compounds that are formed during the sugar degradation process (Figure 6) and that make up some of the potent odorants in the flavor profile were studied (Table II). These compounds are caramellic and jam-like in flavor and aroma and describe the brown/fruity notes perceived by the sensory panelling. Using area count

Downloaded by UNIV OF GUELPH LIBRARY on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch006

Downloaded by UNIV OF GUELPH LIBRARY on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch006

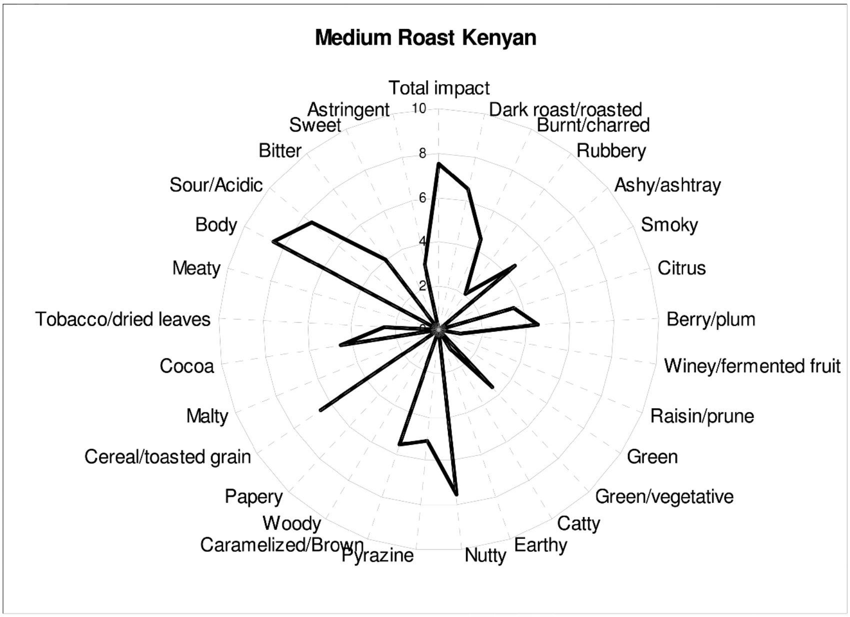


Figure 1. Spider Charts used for sensory profiling.

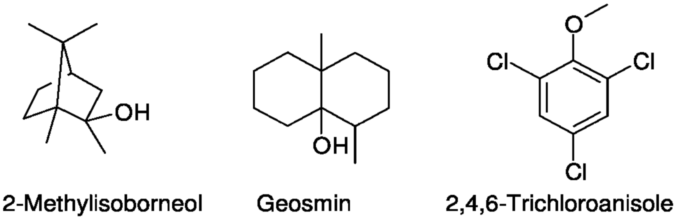


Figure 2. Negatively perceived contributors in Robusta coffee.

comparisons, the Medium Roast Kenyan brewed coffee had approximated seven times the amount of these potent odorants. The use of area counts is by no means a quantitative measurement, but they do qualitatively compare samples as long as all conditions for brewing and extracting remain the same among varietals.

The Kenyan brewed coffee showed a complexity unlike the other Arabica beans. The panelists found the Kenyan brew to have significant citrus notes to its flavor profile. This linked well with analytical results because the mainly terpene compounds that give the citrus notes were found at higher concentrations. For example, compounds including limonene, linalool, linalool oxide furan, terpinolene, p-menth-1-en-9-al, and 6-methyl-5-hepten-2-one were found in the Kenyan brewed coffee at higher levels compared to that of other Arabica beans (results not shown).

There were also subtleties to the Kenyan brew that appeared to enhance its characteristics compared to that of the other varietals (Figure 7).

Downloaded by UNIV OF GUELPH LIBRARY on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch006

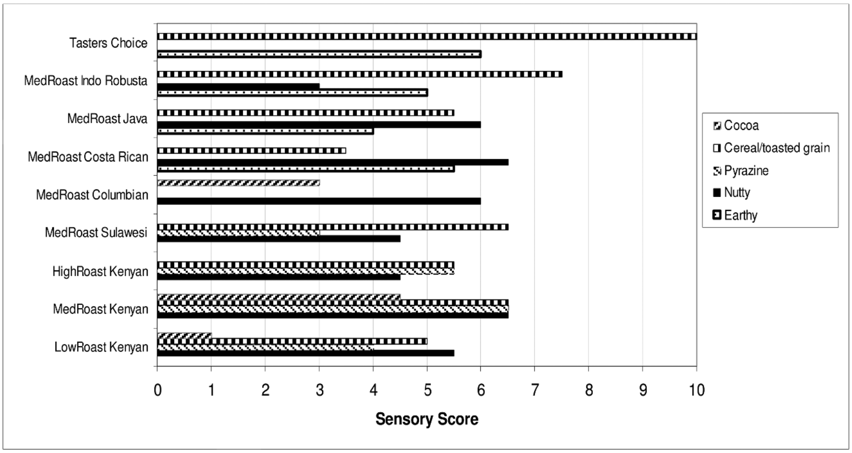


Figure 3. Comparison of "Pyrazine" notes.

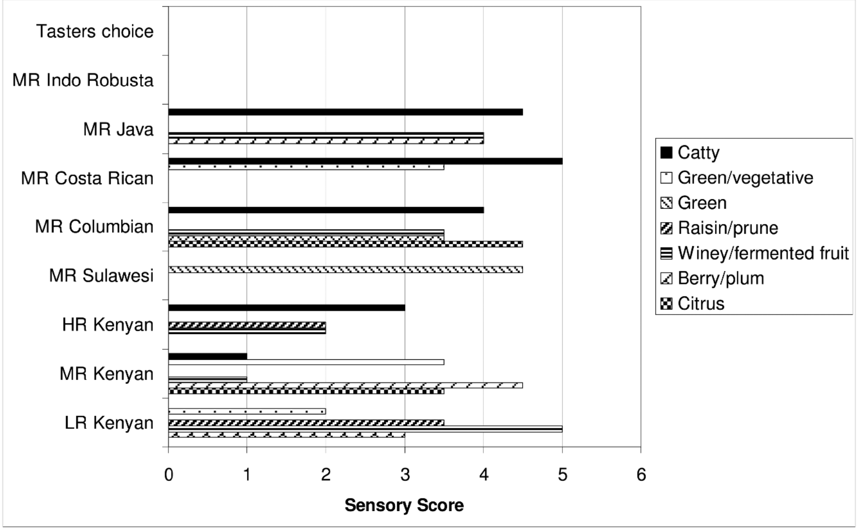


Figure 4. Comparison of "Winey/Berry" notes.

Examples include 2-methyl-5-isopropenyl-2-vinyltetrahydrofuran (herboxide), 3,5,5-trimethyl-4-(2-butenylidene)-2-cyclohexenone (megastigmatrienone), and 3,4-dimethyl-cis-5-pentylidene-2,5-dihydrofuran-2-one (cis-bovolide). Herboxide has a herbaceous, green, citrus, and piney impression and has been found in both tea and hibiscus extracts (unpublished). Megastigmatrienone has sweet tobacco, dried fruit, and honey-like characteristics and has been found in peaches and tobacco (unpublished). Finally, cis-bovolide has notes of celery, floral, green and jasmine and has been found in green pepper, sugar cane, and tea extracts (unpublished). The panellists were able to pick up on the small subtleties,

Downloaded by UNIV OF GUELPH LIBRARY on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch006

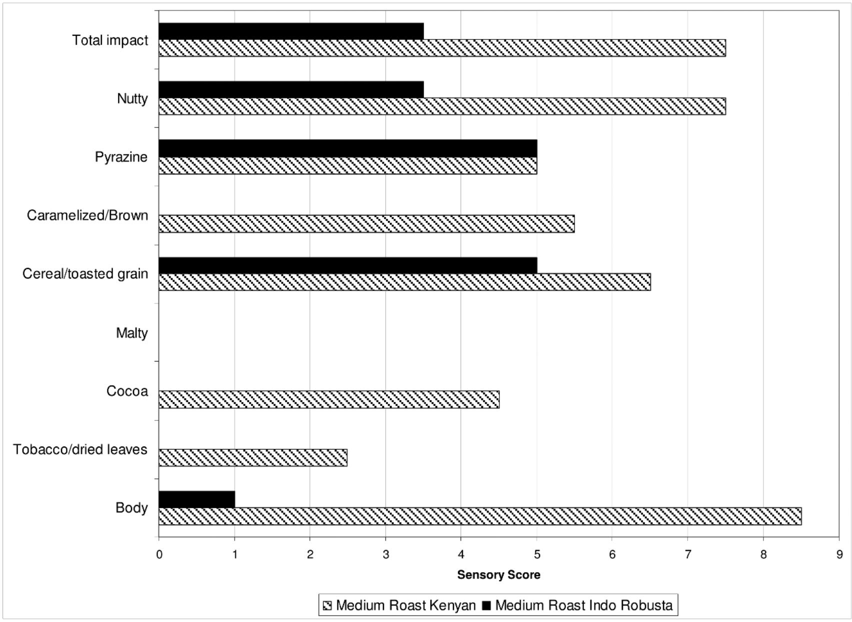


Figure 5. Sugar degradation product descriptors used to compare the Medium Roast Kenyan and Medium Roast Indo Robusta beans.

Table II. Area Count Comparison of Sugar Degradation Products in the Liquid-Liquid Extraction of the Medium Roast Kenyan and Medium Roast Indo Robusta (nd = not detected)

Component	*Kenya (Area Counts)*	*Robusta (Area Counts)*
1-(2-Furyl)-(*E*)-1-buten-3-one	28,520	nd
4,5-Dimethyl-3-hydroxy-2(5h)-furanone	15,550	nd
2-Hydroxy-3-methyl-2-cyclopenten-1-one	166,410	nd
3-Ethyl 2-hydroxy-2-cyclopenten-1-one	91,400	nd
2,5-Dimethyl-4-hydroxy-3(2H)-furanone	533,930	19,530
2-Ethyl-4-hydroxy-5-methyl-3(2H)-furanone	5,814	nd
5-Ethyl-4-hydroxy-2-methyl-3(2H)-furanone	45,350	nd
3-Hydroxy-2-methyl-4(4H)-pyranone	638,900	203,830
Total Area Counts	1,520,060	223,360

which helped to elevate the flavor of the Kenyan coffee to the gold standard among the chosen Arabica beans.

The final objective deals with the findings of both analytical and sensory measurements for the burnt and dark notes of the brewed coffee. From the sensory

Downloaded by UNIV OF GUELPH LIBRARY on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch006

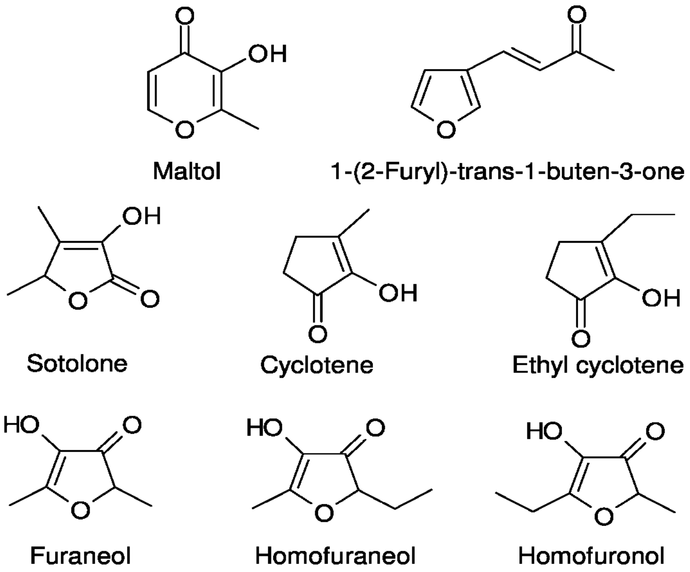


Figure 6. Coffee sugar degradation products.

paneling, it was evident that the burnt and dark notes increased as the roasting temperature of the Kenyan bean increased (Figure 8).

According to literature, pyridine and its derivatives can give a burnt and dark impression to brewed coffee (*1*). Therefore, the precursor to the pyridine derivatives was chosen to be monitored in the three different roasting temperatures of the Kenyan brewed coffee types to see if links could be made to the sensory findings.

Using high performance liquid chromatography (HPLC), measurements were made on a well-known bitter component of coffee called trigonelline (Figure 9) for all three of the roasting temperatures of the Kenyan bean.

Upon roasting, this compound thermally degrades to form pyridine and pyridine derivatives through a non-decarboxylative mechanism (*1*, *13*, *14*). As predicted, the concentration of trigonelline decreased more than two-fold from Low Roast (215°C) to High Roast (245°C). (Table III).

This data suggests that the concentration of pyridine and its derivatives would increase in the volatile analyses. The following derivatives from the breakdown of trigonelline were studied: pyridine; 2-methylpyridine; 3-methylpyridine; 3-ethylpyridine; 4-ethylpyridine; nicotinic acid (niacin); methyl nicotinate.

This result was confirmed as the area counts increased as the roasting temperature of the bean increased (Table IV). This increase in area counts from the Low Roast to the Medium Roast Kenyan is not as high as would be expected especially from the trigonelline drop-off, but it is an increase none the less. Similar results

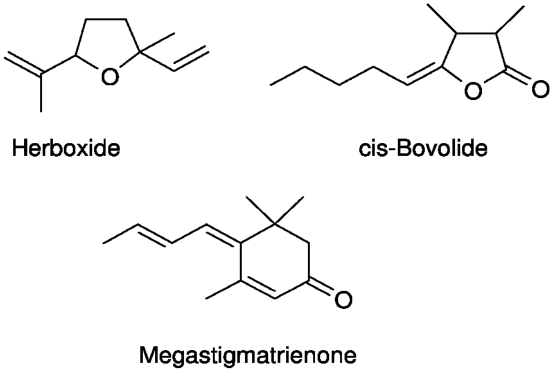


Figure 7. Compounds of interest in Kenyan brew coffee.

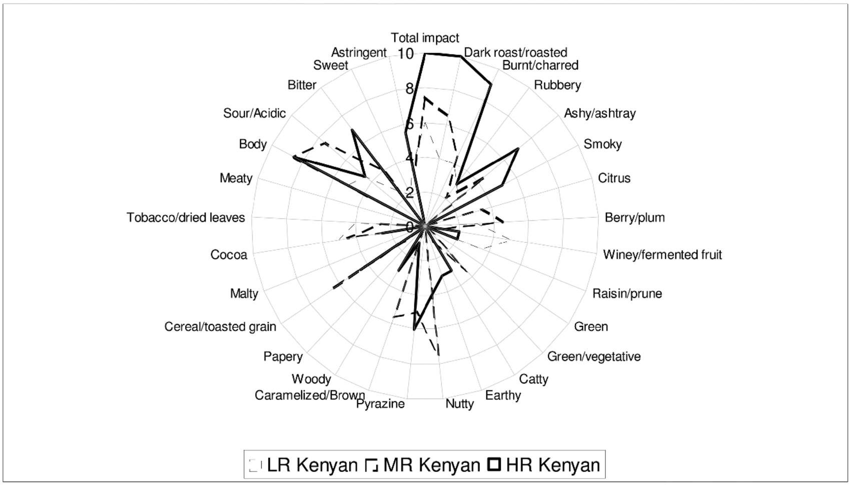


Figure 8. Comparison of the Roasting temperatures for Kenyan (Arabica) bean (LR = Low Roast; MR = Medium Roast; HR = High Roast).

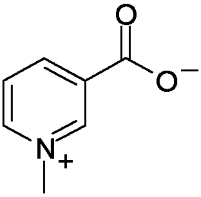


Figure 9. Structure of Trigonelline.

were discovered for the other nitrogen heterocyclic compounds, including the pyrrole and pyrazine derivatives (results not shown).

Downloaded by UNIV OF GUELPH LIBRARY on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch006

Downloaded by UNIV OF GUELPH LIBRARY on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch006

Table III. Trigonelline Concentration in the Three Roasting Temperatures of the Kenyan Bean

Compound	*Kenyan 215°C Concentration ppm (µg compound/g sample)*	*Kenyan 230°C Concentration ppm (µg compound/g sample)*	*Kenyan 245°C Concentration ppm (µg compound/g sample)*
Trigonelline	13,440	10,218	6,383

Table IV. Area Count Comparison of Pyridine and Pyridine Derivatives for the Three Roasting Temperature of the Kenyan Bean

	Kenya 215°C (Area Counts)	*Kenya 230°C (Area Counts)*	*Kenya 245°C (Area Counts)*
Total Area Counts for Pyridine and Pyridine Derivatives	30,810	31,830	300,190

Conclusion

Important links between analytical and sensory data were made through this coffee research project. The different roasts of Arabica beans exhibited both aroma and flavor differences, specifically related to the pyrazines and winey/berry notes. Analytical results confirmed these differences. The Arabica and Robusta beans were also compared, and results from both analytical and sensory measurements showed differences between the pyrazines and the sugar degradation products. The Robusta beans have relatively higher area counts for the pyrazine compounds compared to that of the Arabica beans, which explains the panelists perception of more earthy and woody aromas. On the other hand, the sugar degradation products were found at high area counts for the Arabica beans, which linked to sensory and gave a higher caramel, sweet-like aroma to the brewed coffee. The final study dealt with the flavor differences in roasting temperature for the Kenyan bean. Results showed that the nitrogen heterocyclic components increased in number and concentration as the roast temperature increased. These nitrogen heterocyclic compounds tended to give the more desirable burnt, dark, and ashtray-like notes to the brewed coffee. Sensory panels aided in linking these findings. The High Roast Kenyan bean had much higher levels of these particular notes, compared to that of the Medium and Low Roasted beans.

References

1. Flament, I. *Coffee Flavor Chemistry*; John Wiley & Sons, Ltd.: England, 2002.
2. Clark, R. J.; Vitzthum, O. G. *Coffee Recent Developments*; Blackwell Science Ltd: Oxford, 2001

3. Lindinger, C.; Labbe, D.; Pollien, P.; Rytz, A.; Juillerat, M. A.; Yeretzian, C.; Blank, I. When machine tastes coffee: Instrumental approach to predict the sensory profile of espresso coffee. *Anal. Chem.* **2008**, *80*, 1574–1581.
4. Mayer, F.; Czerny, M.; Grosch, W. Influence of provenance and roast degree on the composition of potent odorants in Arabica coffees. *Eur. Food Res. Technol.* **1999**, *209*, 242–250.
5. Hwang, H. I.; Hartman, T. G.; Ho, C. T. Relative Reactivites of Amino Acids in Pyrazine Formation. *J. Agric. Food Chem.* **1995**, *43*, 179–184.
6. Keyhani, A.; Yaylayan, V. A. Elucidation of the mechanism of pyrazinone formation in glycine model systems using labeled sugars and amino acids. *J. Agric. Food Chem.* **1996**, *44*, 2511–2516.
7. Cantergiani, E.; Brevard, H.; Krebs, Y.; Feria-Morales, A.; Amadó, R.; Yeretzian, C. Characterization of the aroma of green Mexican coffee and identification of moldy/earthy defect. *Eur. Food Res. Technol.* **2001**, *212*, 648–657.
8. Blank, I.; Grosch, W. On the role of (-)-2-methylisoborneol for the aroma of Robusta Coffee. *J. Agric. Food Chem.* **2002**, *50*, 4653–4656.
9. Bell, G. A.; Laing, D. G.; Panhuber, H. Odor mixture suppression – evidence of a peripheral mechanism in human and rat. *Brain Res.* **1987**, *426* (1), 8–18.
10. Jinks, A.; Laing, D. G.; Hutchinson, I. A psychophysical study of the relationship between temporal processing in odor mixtures and transduction pathways. *Cognit. Brain Res.* **2001**, *12* (2), 273–288.
11. Laing, D. G. Coding of chemosensory stimulus mixtures. *Ann. N. Y. Acad. Sci.* **1987**.
12. Laing, D. G.; Link, C.; Jinks, A. L.; Hutchinson, I. The Limited capacity of humans to identify the components of taste mixtures and taste-odour mixtures. *Perception* **2002**, *31* (5), 617–635.
13. Rizzi, G. P.; Sanders, R. A. Mechanism of pyridine formation from trigonelline under coffee roasting conditions. *Spec. Publ. R. Soc. Chem.* **1996**, *197*, 206–210.
14. Viani, R.; Horman, I. Thermal behavior of trigonelline. *J. Food Sci.* **1974**, *39*, 1216–1217.

Downloaded by UNIV OF GUELPH LIBRARY on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch006

Chapter 7

Aroma Components of Fresh and Stored Pomegranate (*Punica granatum L.*) Juice

Keith R. Cadwallader,[*] Lauren C. Tamamoto, and Stephanie C. Sajuti

University of Illinois, Department of Food Science and Human Nutrition,
1302 W. Pennsylvania Ave., Urbana, IL 61801, USA
[*]cadwlldr@illinois.edu

Downloaded by DUKE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch007

Aroma components of the fresh and stored pomegranate juices were compared. Volatiles were isolated by direct solvent extraction - solvent assisted flavor evaporation (DSE-SAFE) and identified by gas chromatography-olfactometry (GCO), aroma extract dilution analysis (AEDA) and GC-mass spectrometry (GC-MS). Green, fruity, floral and earthy aroma notes and sour, sweet and astringent tastes/mouthfeel factors were indicated by sensory descriptive analysis. Predominant odorants in fresh pomegranate juice included hexanal, (Z)-3-hexenal, 1-octen-3-one, 2-isopropyl-3-methoxypyrazine, 3-(methylthio)-propanal (methional), β-damascenone, *trans*-4,5-epoxy-(*E*)-2-decenal and o-aminoacetophenone. Storage of the fresh juice caused decreases in hexanal and (Z)-3-hexenal, and increases in (*Z*)-3-hexenol, methional, 2-isopropyl-3-methoxy-pyrazines and ethyl cinnamate. The decline in compounds with intense green notes (e.g. hexanal and (*Z*)-3-hexenal) could explain why the green aroma attribute was scored at a lower intensity in the stored juice.

Introduction

Pomegranate (*Punica granatum L.*) is native to tropical and subtropical areas, including countries in the Mediterranean and Central and Western Asia. The fruit is 7-12cm in diameter, with a tough rind and center filled with fruit arils (seed casings) which are surrounded by membranes of spongy tissue. Depending on

In Flavors in Noncarbonated Beverages; Da Costa, N., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

the variety of pomegranate, the color of the flesh ranges from yellow to deep red/purple. In the US the “Wonderful” cultivar is most commonly grown and processed and is known for its deep purple colored flesh (*1*).

In the past, pomegranates have been used as decorative pieces as well in culinary applications such as jams, jellies, sauces and grenadine syrup. Pomegranate-flavored products have grown in popularity in recent years and are now sold as beverages, chewing gums, candies and frozen fruit bars. The commercial popularity of the pomegranate stems from media attention focused on the numerous health benefits of the fruit. Pomegranate fruits are full of vitamins A, C and E, and the deep purple flesh and juice of the fruit contains polyphenols such as anthocyanins, tannins, and ellagic acid which are known to have beneficial antioxidant properties. Researchers believe that pomegranate juice contains more antioxidant activity than red wine and green tea (*2*). The consumption of pomegranate juice has also been shown to inhibit lipoprotein oxidation and to reduce free radicals (*2*, *3*).

Research has been conducted on the physiochemical composition of different varieties of pomegranate (*4*), and on the properties of pomegranate at different maturation stages (*5*). Previous studies have also examined the quality of pomegranate juice as affected by various processing (*1*) and storage methods (*6*, *7*), and after removal of cloudy phenolic compounds (*8*). Raisi *et al* (*9*) evaluated the effects of pervaporation on the recovery of four volatile compounds in pomegranate juice. Atsushi and Katsumi (*10*) identified 75 and 116 volatile compounds in the seeds (arils) and rind of pomegranate, respectively. Through the use of gas chromatography-olfactometry and aroma extract dilution analysis, these researchers also demonstrated the importance of (Z)-3-hexenal, (Z)-3-hexenol, 2-aminoacetophenone, β-damscenone, 3-(methylthio)-propanal (methional) and 2-isopropyl-3-methoxypyrazine in overall pomegranate aroma.

Additional studies on the aroma compounds of pomegranate and effect of storage are needed to precisely determine the compounds that contribute to the unique flavor of pomegranate juice.

Downloaded by DUKE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch007

Experimental

Pomegranates

Fresh POM Wonderful fruit (POM Wonderful LLC., Los Angeles, CA) was grown in San Joaquin Valley, CA and shipped to Urbana, IL and stored at room temperature (~20°C) until juice extraction. Solvents and authentic flavor standards in Table II were obtained from Sigma-Aldrich (St. Louis, MO), except for no. 17 which was obtained from Firmench (Princeton, NJ) and no. 20 which was synthesized using a previously published procedure (*11*).

Downloaded by DUKE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch007

Juice Preparation

Pomegranate fruit was rinsed with deodorized distilled water and then cut in half. The arils of pomegranate were extracted using an electric juicer (Black and Decker "Handy Juicer"). The juice was then filtered through a nylon mesh bag and then centrifuged for 10 minutes at 3,000rpm in 250mL PTFE bottles. The juice was divided and half was stored in a glass container at 4°C for 24 hr, while the remaining "fresh" juice was immediately subjected to the volatile isolation procedure described below.

Titratable Acidity (TA) and Soluble Solids Content (SSC)

The SSC of the juice was measured using a refractometer. Citric Acid is the predominant acid in pomegranate juice (*12*) and was used for the calculation of acid content of the juice, which was determined by potentiometric (pH 8.1) titration of 10mL of juice with 0.1 N NaOH (*4*).

Isolation of Volatile Components

Juice (125mL) was extracted with diethyl ether (3 x 70mL). The ether extracts were pooled and concentrated to about 100 mL by a gentle distillation using a Vigreux column at 43°C and then subjected to solvent-assisted flavor evaporation (SAFE) as previously described (*13*). The SAFE distillate (aroma extract) was dried over anhydrous sodium sulfate (10g), concentrated to 10mL using the aforementioned gentle distillation procedure and then further concentrated to 200µL under a gentle stream of nitrogen. Extracts were stored at -70°C in 2mL vials equipped with PTFE-lined caps until analysis.

Sensory Evaluation

Descriptive sensory analysis was conducted by a trained eight person panel. Each panelist had more than 20 hr or formal sensory training and received an additional 5 hr of training with pomegranate juice where they defined sensory terms and references for the aroma and taste/mouthfeel attributes. Terms and references are given in Table I. Aroma attributes were evaluated orthonasally using 125mL PTFE squeeze bottles containing 10mL of juice. For evaluation of taste attributes, juice was presented in 5oz plastic cups with lids. Panelists were instructed to score the aroma and taste attributes using a 15-point universal scale, where 0 = none and 15 = very strong (*14*). Other procedural details and statistical analyses have been previously described (*13*, *15*).

Instrumental Analysis

For gas chromatography-olfactometry (GCO) and GC-mass spectrometry (GC-MS) the aroma extracts (2µL) were analyzed using cool on-column injection. All other details regarding the apparatus and methods used for GCO, GC-MS and aroma extract dilution analysis (AEDA) have been previously described (*13*). Compounds were identified based on the comparison of retention indices determined against n-alkanes on two columns, electron-impact mass spectra and odor characteristics (GCO) against those of authentic standard compounds.

Results and Discussion

Composition and Sensory Characteristics of Pomegranate Juice

The fresh juice had an average soluble solids content (SSC) of 16.13 ± 0.46 °Brix and a titratable acidity of 1.13 ± 0.03g/100mL (based on citric acid). These values are within the normal range expected for ripe pomegranates (*4*, *16*, *17*). Melgarejo *et al* (*16*) used average total acidity (TA) to group forty Spanish pomegranate cultivars into three categories, namely sweet (~ 0.3g/100mL TA), sweet-sour (~ 0.8g/100mL TA), and sour (~ 2.7g/100mL TA). The pomegranate cultivar evaluated in the present study could be grouped among the sour sweet group based on its TA.

Four aroma terms and three taste/mouthfeel attributes were identified by sensory descriptive analysis. Terms, references and ratings determined for the references are listed in Table I. In preliminary sensory experiments a noticeable change was observed in the aroma quality of the juice after one day of refrigerated storage. This was considered a potentially important observation since the industry often stores the freshly pressed juice prior to pasteurization. For this reason, a comparison of the sensory profiles of the fresh and stored pomegranate juice was conducted. The results are shown in Figure 1. A green note, followed by fruity and floral notes, were present at highest intensities in both fresh and stored pomegranate juices. A faint earthy aroma was also detected in both juices. The predominant taste/mouthfeel attributes were sweet, sour and astringent. The intensities of most of the sensory attributes were not changed after refrigerated storage, except for the green aroma note which was scored at a lower intensity ($p \leq 0.05$) in the stored juice.

Predominant Odorants in Fresh and Stored Juices

Volatile components of intermediate and low volatility were isolated from the pomegranate juices by direct solvent extraction followed by a mild SAFE cleanup step to remove nonvolatile material from the extracts. The 'clean' aroma extracts were then analyzed by cool on-column injection-GCO, which helped to minimize

Downloaded by DUKE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch007

Table I. Sensory Descriptive Terms and References

Term	*Standard reference*	*Rating*[a]
Aroma[b]		
Green	10mL of 50ppm of (E)-2-hexenal	7
Floral	10mL of 50ppm of phenylacetaldehyde	8
Fruity	10mL of 2ppm of ethyl butyrate	6
Earthy	6.5g of russet potato peels	3
Taste/mouthfeel[c]		
Astringent	15mL of 0.2% tannic acid	5
Sour	15mL of 0.5% citric acid solution	10
sweet	15mL of 10% sucrose solution	10

[a] Ratings for references were based on a 15 point universal scale, where 0 = none and 15 = very. [b] For aroma analyses, samples were evaluated orthonasally in 12mL PTFE squeeze bottles. [c] For taste evaluations, samples were presented in Solo cups.

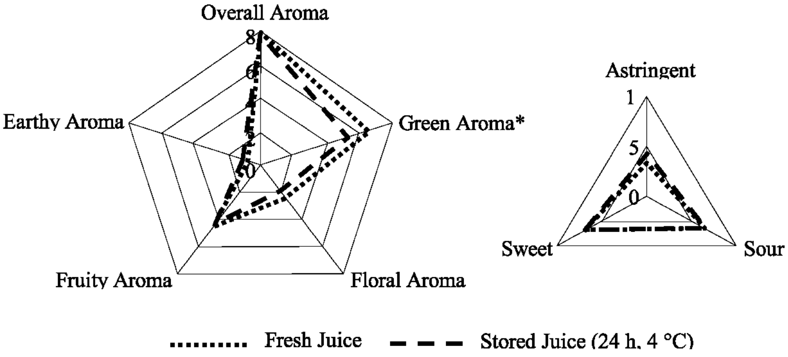


*Figure 1. Descriptive sensory profile of fresh and stored pomegranate juice (*indicates significant difference, p≤0.05).*

any potential loss or degradation of the characteristic aroma components of the juices.

Twenty-three odorants with FD factors ≥ 3 were detected by GCO and AEDA (Table II). Results of AEDA were in good general agreement with the sensory evaluation results. The major odorants identified included those with green notes (nos. 3-5, 10, 16, 18 and 20), fruity notes (nos. 1, 2, 8, 21 and 23), floral notes (nos. 15 and 19) and earthy notes (nos. 9, 11, 12, 14). The three remaining odorants not mentioned above imparted malty (no. 6), minty (no. 7) and clove-like (no. 22) notes to the juices.

Downloaded by DUKE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch007

Downloaded by DUKE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch007

Table II. Odorants Detected by Aroma Extract Dilution Analysis of Fresh and Stored Pomegranate Juice

			Retention index[b]		*Fd-factor*[c]	
No.	*compound*	*Odor description*[a]	*WAX*	*RTX5*	*Fresh*	*Stored*
1	Ethyl 2-methylbutyrate	Fruity	1044	849	3	3
2	Ethyl 3-methylbutyrate	Fruity, berry	1060	852	9	9
3	Hexanal	Green, cut-grass	1084	801	27	<3
4	Unknown	Green, sweaty	1139	- - [d]	9	<3
5	(*Z*)-3-Hexenal	Green, pungent, cut-leaf	1146	1029	243	3
6	2-Methyl-(*E*)-2-pentenal	Sour, bug, malty	1161	- -	9	3
7	1,8-Cineole	Minty, eucalyptus	1202	1029	3	3
8	Ethyl hexanoate	Fruity, green apple	1227	1002	3	3
8	Octanal	Orange	1293	1001	<3	3
9	1-Octen-3-one	Mushroom, earthy	1297	975	27	3
10	(*Z*)-3-Hexenol	Green, cut-leaf	1389	857	<3	9
11	2-isoPropyl-3-methoxypyrazine	Earthy, soil	1427	1095	81	243
12	3-(Methylthio)-propanal (Methional)	Potato, earthy	1451	905	27	81
14	2-isoButyl-3-methoxypyrazine	Earthy, bell pepper	1517	1182	3	3
15	Phenylacetaldehyde	Rosy, plastic	1646	1049	9	3
16	Unknown	Melon, hay, stale	1714	- -	9	9
17	β-Damascenone	Applesauce, floral	1824	1390	27	27
18	Unknown	Unripe, green, fatty	1875	- -	27	27
19	2-Phenylethanol	Floral, rosy, wine-like	1913	1120	<3	9
20	trans-4,5-Epoxy-(*E*)-2-decenal[e]	Metallic, unripe, green	2006	1380	81	27
21	Ethyl 3-phenyl-(*E*)-2-propenoate (Ethyl cinnamate)	Grape, candy, fruity	2130	1469	27	243
22	4-Allyl-2-methoxyphenol (Eugenol)	Spicy, cloves	2159	1360	9	9
23	*o*-Aminoacetophenone	Grape, musky, fruity	2225	1299	81	81

[a] Odor quality as perceived during GCO. [b] Retention indices were calculated rom GCO data, WAX = Stabilwax column. [c] Flavor dilution (FD) factor determined on Sabilwax column. [d] - - = not available. [e] Compound tentatively identified based on comparison of its odor property and retention indices with reference compound.

Downloaded by DUKE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch007

Refrigerated storage altered the aroma components of the pomegranate juice. In fresh juice, (*Z*)-3-hexenal (no. 5), 2-isopropyl-3-methoxypyrazine (no. 11), *trans*-4,5-epoxy-(*E*)-2-decenal (no. 20) and *o*-aminoacetophenone (no. 23) had the greatest impact (FD factors ≥ 81) on the overall aroma, followed by hexanal (no. 3), 1-octen-3-one (no. 9), 3-(methylthio)-propanal (methional, no. 12), β-damascenone (no. 17) and ethyl cinnamate (no. 22). On the other hand, 2-isopropyl-3-methoxypyrazine (no. 11), ethyl cinnamate (no. 22), 3-(methylthio)-propanal (no. 12) and *o*-aminoacetophenone (no. 23) had greatest impact (FD factors ≥ 81) on the overall aroma of the stored pomegranate juice.

Specific changes that occurred during storage included increases in octanal (no. 8), (*Z*)-3-hexenol (no. 10), 2-isopropyl-3-methoxypyrazine (no. 11), methional (no. 12), 2-phenylethanol (no. 19), ethyl cinnamate (no. 21), and decreases in hexanal (no. 3), (*Z*)-3-hexenal (no. 5), 2-methyl-2-pentenal (no. 6), 1-octen-3-one (no. 9) , phenylacetaldehyde (no. 15) and trans-4,5-epoxy-(*E*)-2-decenal (no. 20).

Our results in good agreement with those of Atsushi and Katsumi (*10*) who demonstrated by application of AEDA that (*Z*)-3-hexenal, (*Z*)-3-hexenol, o-aminoacetophenone, *β*-damascenone and methional had the highest FD-factors in pomegranate fruit (arils). They further reported that the rind of the fruit contained 2-isopropyl-3-methoxypyrazine which contributed an earthy note to the fruit.

In the present study, the decreases in the potent green odorants (nos. 3 and 5) could explain the significant decline in the green character of the stored juice (Figure 1). The development of hexanal and (*Z*)-3-hexenal in the fresh juice was probably due to lipoxygenase action on linoleic and linolenic acids, respectively (*18*). These two compounds, along with nos. 6, 9, 15 and 20 could have been subsequently converted to their corresponding alcohols by a nonspecific aldehyde dehydrogenase (*18*). The net effect was that stored pomegranate juice exhibits a less green character than the fresh juice.

Conclusions

A juice extraction method was developed in which juice was obtained primarily from the arils of the pomegranate fruit. Pomegranate fruit has some key aroma compounds that are responsible for its green, fruity, floral and earthy character. Characteristic aroma components of pomegranate juice also appeared to change upon refrigerated storage. Additional research should be conducted to quantify the aroma components in pomegranate as affected by processing and storage. With regards to processing, the flavor components of the pomegranate juice might differ if the juice was pressed from the whole fruit (rind and flesh) versus only from the fleshy arils. Therefore, more research should be conducted on the flavor components that are contributed by the rind of the pomegranate. The effects of pasteurization and storage should also be examined to determine the impacts of these factors on the flavor quality of pomegranate juice.

Downloaded by DUKE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch007

Acknowledgments

The authors express their thanks to Mr. Miguel Santos from Paramount Fruit and POM Wonderful, LCC for the generous donation of the POM Wonderful fruit used in this project.

References

1. Hess-Pierce, B.; Kader, A. A. Responses of ‘Wonderful’ pomegranates to controlled atmospheres. *Acta Hortic.* **2003**, *600*, 751–757.
2. Gil, M. I.; Tomas-Barberan, F. A.; Hess-Pierce, B.; Holcroft, D. M.; Kadar, A. A. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *J. Agric. Food Chem.* **2000**, *48*, 4581–4589.
3. Aviram, M.; Rosenblat, M.; Gaitini, D. Pomegranate juice consumption for 3 years by patients with carotid artery stenosis reduces common carotid intima-media thickness, blood pressure and LDL oxidation. *Clin. Nutr.* **2004**, *23*, 423–433.
4. Fadavi, A.; Barzegar, M.; Azizi, M. H.; Bayat, M. Note. Physiochemical composition of ten pomegranate cultivars (*Punica granatum* L.) grown in Iran. *Food Sci. Technol. Int.* **2005**, *11*, 113–119.
5. Al-Maiman, S. A.; Ahmad, D. Changes in physical and chemical properties during (*Punica granatum* L.) fruit maturation. *Food Chem.* **2002**, *76*, 437–441.
6. Miguel, G.; Dandlen, S.; Antunes, D.; Neves, A.; Martins, D. The effect of two methods of pomegranate (*Punica granatum* L) juice extraction on quality during storage at 4 °C. *J. Biomed. Biotechnol.* **2004**, *5*, 332–337.
7. Lopez-Rubira, V.; Conesa, A.; Allende, A.; Artes, F. Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C. *Postharvest Biol. Technol.* **2005**, *37*, 174–185.
8. Alper, N.; Acar, J. Removal of phenolic compounds in pomegranate juices using ultrafiltration and laccase-ultrafiltration combinations. *Nahrung* **2004**, *48*, 184–187.
9. Raisi, A.; Aroujalian, A.; Kaghazchi, T. Multicomponent pervaporation process for volatile aroma compounds recovery from pomegranate juice. *J. Membr. Sci.* **2008**, *322*, 339–348.
10. Atsushi, Y.; Katsumi, U. Volatile constituents of pomegranate (*Punica granatum* L.). *Koryo, Terupen oyobi Seiyu Kagaku ni kansuru Toronkai Koen Yoshishu* **2000**, *44*, 1–3.
11. Lin, J.; Fay, L. B.; Welti, D. H.; Blank, I. Synthesis of *trans*-4,5-epoxy-(*E*)-2-decenal and its deuterated analog used for the development of a sensitive and selective quantification method based on isotope dilution assay with negative chemical ionization. *Lipids* **1999**, *34*, 1117–26.

12. Nelson, E. K. The non-volatile acids of the pear, quince, apple, loganberry, blueberry, cranberry, lemon and pomegranate. *J. Am. Chem. Soc.* **1927**, *49*, 1300–1302.
13. Watcharananun, W.; Cadwallader, K. R.; Huangrak, K.; Kim, H.; Lorjaroenphon, Y. Identification of predominant odorants in Thai desserts flavored by smoking with “tian op”, a traditional Thai scented candle. *J. Agric. Food Chem.* **2009**, *57*, 996–1005.
14. Meilgaard M.; Civille, G. V.; Carr, B. T. *Sensory Evaluation Techniques*, 3rd ed; CRC Press LLC: Boca Raton, FL, 1999.
15. Zhou, Q.; Wintersteen, C. L.; Cadwallader, K. R. Identification and quantification of aroma-active components that contribute to the distinct malty flavor of buckwheat honey. *J. Agric. Food Chem.* **2002**, *50*, 2016–2021.
16. Melgarejo, P.; Salazar, D. M.; Artes, F. Organic acids and sugars composition of harvested pomegranate fruits. *Eur. Food Res. Technol.* **2000**, *211*, 185–190.
17. Poyrazoglu, E.; Gokmen, V.; Artik, N. Organic acids and phenolic compounds in pomegranates (*Punica granatum* L.) grown in Turkey. *J. Food Compos. Anal.* **2002**, *15*, 567–575.
18. Hatanaka, A.; Harada, T. Formation of *cis*-3-hexenal, *trans*-2-hexenal and *cis*-3-hexenol in macerated *Thea sinensis* leaves. *Phytochemistry* **1973**, *12*, 2341–2346.

Downloaded by DUKE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch007

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch008

Chapter 8

Evaluation of Apple Juice Aroma

The Aroma Index as a Model for the Objective Description of Aroma Restoration

Carolin Wolter,[1,*] Achim Gessler,[2] and Peter Winterhalter[1]

**[1]Institute of Food Chemistry, Technische Universität Braunschweig,
Schleinitzstraße 20, DE-38106 Braunschweig, Germany
[2]Wesergold Getränkeindustrie GmbH & Co KG, Behrenstraße 44- 64,
DE-31737 Rinteln, Germany
[*]cwolter.qm@wesergold.de**

On the basis of comprehensively researched data an exemplary aroma index model was created. This model is supposed to ensure an objective description of aroma restoration in apple-juice from concentrate and may in modified variations also be transferable to further sorts of fruit juices. The aroma index (for apple juice) includes various types of aroma compounds occurring in apple juice and contributing to its typical flavor. The fruity smelling esters are taken into account as well as the green-grassy smelling compounds like carbon-6-aldehydes and –alcohols and some as fruity-aromatic smelling described alcohols. By considering miscellaneous, in diverse bio-chemical pathways formed substances, seasonal and cultivar-depending variations can be adjusted without claiming a special standardized aroma profile.

Introduction

According to the European Council directive 2001/112/EC fruit juice from concentrate is defined as “the product obtained by replacing, in the concentrated fruit juice, water extracted from that juice during concentration. Thus restoring the flavors, and, if appropriate, pulp and cells lost from the juice but recovered during the process of producing the fruit juice in question or of fruit juice of the

In Flavors in Noncarbonated Beverages; Da Costa, N., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

same kind." The product thus obtained must display organoleptic and analytical characteristics at least equivalent to those of an average type of juice obtained from fruits of the same kind not from concentrate (*1*).

In order to ensure an adequate restoration of fruit juice from concentrate more and more analytical methods, in addition to the established organoleptic tests, are deployed to evaluate not only the main chemical parameters as brix and acidity, but also the minor components. This includes aroma active compounds, which are often contained only in trace amounts (ppb range). The authenticity test of fruit juice aroma, using enantioselective capillary gas chromatography, developed in the 1980′s (*2–4*), has proven to be an important analytical technique and is common practice nowadays. In addition to this authenticity check, the recently brought up evaluation of sufficient (re-)aromatization of fruit juices from concentrate by means of contents of particular aroma compounds is expected to offer a further guideline for assessment of correct manufacturing practice. The absolute aroma content shall be judged and a minimum requirement for aroma restoration set. This approach will offer a way to reveal insufficiently re-aromatized fruit juices. It must not be seen as a quality standard-setting, which due to natural or technological circumstances would possibly discriminate against some on the lower level aromatized, but still regularly produced products. For all this the limit-setting has to be well-reasoned.

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch008

Preliminary Considerations

Various ideas and models for the quantitative evaluation of fruit juice aroma have been suggested over the last years. Heil and Ara (*5*) used the "sum of esters (excluding butyl acetate)" as a possible evaluation model for apple juice from concentrate. Like many other authors (*6–11*) they describe the esters as an important class for the typical fruity apple-juice aroma. From the sum total of esters the content of butyl acetate is subtracted because otherwise its concentration would by far dominate the parameter "sum of esters". On the basis of data gathered in their analyses of various juices, Heil and Ara (*5*) proposed a "sum of esters excluding butyl acetate" of 150µg/L as useful minimum guideline. They discussed the inclusion of carbon-6-aldehydes and –alcohols, as these make an important contribution to the "green" odor impression of apple juice.

But so far no limit has been set for those aroma substances. On the other hand Hey et al. (*11*) came to the conclusion that the variability in the qualitative and quantitative composition of apple juice aroma excluded the development of a pertinent AIJN-reference guideline or RSK values. Both the Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Union (AIJN) and the German committee for guidelines and ranges of variation of certain parameters in fruit juices (RSK value committee) have set up guidelines with absolute requirements and quality parameters on fruit juices to verify identity and authenticity.

Before developing our own concrete model for evaluating apple juice aroma, requirements for a reliable model were defined:

- The aimed model must not discriminate against correctly produced products.
- It shall consider important aroma substances contributing to the typical juice aroma and having an indicator function. Substances that cause off-flavors should not be taken into account. The substances should be weighted according to their contribution to the general aroma impression and their common contents in apple juice.
- The model must not discriminate against certain organoleptic character types and shall compensate differences resulting from variety, geographic origin or harvest time.
- With regard to the practicability all substances of the aroma model should be ascertainable with one, yet to be defined, method. Other equivalent methods have to be verified in inter-laboratory tests.

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch008

Development of the Aroma Index

Our evaluation model, the so-called "Aroma Index", has been developed on the basis of data collected in the year 2007 (*12*). With the aim of setting up a model analogous to the GDCh formula to determine fruit juice content (*13*) reference values for certain aroma substances have been established. They are standardized in a weighted formula to generate a dimensionless index number for assessing the aroma content. The model is calculated in such a way that an index value of at least 100 has to be reached for an appropriately re-aromatized juice from concentrate. Products with an index value below 100 should be subjected to further analytical and organoleptic examinations and, if necessary, be classified as insufficiently re-aromatized.

As important components for the apple juice aroma, ten compounds were identified and therefore included in the index model. Among the esters the five compounds ethyl 2-methylbutyrate, ethyl butyrate, 2-methylbutyl acetate, hexyl acetate and butyl acetate were taken into account. Additionally, two carbon-6-aldehydes, hexanal and *(E)*-2-hexenal as well as two carbon-6-alcohols, hexanol and *(E)*-2-hexenol and the aliphatic alcohol 2-methylbutanol were included in the aroma index. 3-Methyl-branched volatiles like isoamyl acetate and isoamyl alcohol are not typical apple juice flavor compounds (*7*) and therefore are not included in the aroma index model.

The choice of these ten compounds was rationalized by their odor threshold values in water (Table I) and their average contents in common apple juices. All of these compounds were typical components of apple juice aroma and were present in nearly all of the tested apple juices. Their low odor threshold values in water, were gathered from several references (*8*, *11*, *14*), and verify their crucial contribution to the typical apple juice aroma. Nevertheless it should be taken into account that odor threshold values may differ in different matrices in ranges up to several orders of magnitude, and the absolute odor threshold values in apple-juice have not been verified until now. It is known that odor threshold values depend on solvent, pH value, and other effects caused by accompanying constituents (*14*).

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch008

Table I. Odor Threshold Values of Selected Apple Juice Aroma Compounds in Water

Aroma Compound	*Odor Threshold Value in water (µg/L)*	*Reference*
Ethyl 2-methylbutyrate	0.13	(*11*)
Ethyl butyrate	0.76	(*8*, *11*)
Hexyl acetate	2	(*11*)
2-Methylbutyl acetate	5	
Butyl acetate	66	(*11*)
Hexanal	2.4	(*8*, *11*)
(E)-2-Hexenol	75	(*11*)
(E)-2-Hexenal	110	(*8*, *11*)
Hexanol	500	(*8*, *11*)
2-Methylbutanol	500	(*11*)

Other odorants of apple juice that show even lower odor threshold values in water, such as, e.g., β-damascenone (0.002µg/L) (*8*), are not taken into consideration as there is no suggestion of them having an indicator function for apple juice aroma restoration. Thus β-damascenone has no suitable criterion for re-aromatization of apple juice from concentrate because of its low volatility. During concentration, the majority of the β-damascenone content remains in the concentrate and does not pass over into the recovered aroma phase. Therefore β-damascenone is not an essential component of apple juice restoration aroma and its content does not provide any information on re-aromatization. Restoration water phases are the aqueous aroma condensates recovered from steam generated by the process of apple juice concentration, and are used for the natural re-aromatization of apple juices from concentrate.

The aim of the aroma index model is to reveal if a tested juice is analytically equivalent to an average type of juice; provided that the organoleptic standards are fulfilled. The average demand of juices made from concentrate can not be the mathematical mean value or median of analyses of “not from concentrate” (NFC) juices, because this would set an inappropriate quality claim, which technologically in the most cases can not be fulfilled. It was shown that considerable amounts of the high volatile aroma substances are lost in technological production processes such as filtration and concentration/aroma recovery (*11*, *12*). These processes are clearly declared to be legally permissible and often necessary to produce products of constant quality. Those inevitable losses have to be taken into consideration when claiming equality with NFC juices. The legally required equality to average NFC juices should not be orientated towards the calculated mathematical mean value or median of NFC juices, but more sensibly on a wide range around the mean value, as proposed in the AIJN reference guidelines and RSK-value tables. The aroma of juices show a much wider variance than most of the main reference parameters of juices such as brix, density, and acidity. For that the purpose, the aromatization range has to be at least as broad as for the established criteria stated in the AIJN-Code of practice.

Amongst the statistics from which the aroma index model was generated, is various analytical data carried out in our own and private food control laboratories respectively, that has been included as well as data from available published studies (*5*, *6*, *11*). The model's reference values should represent all products on sale made in accordance with common industry practices, covering the entire variability of all influencing factors. In order to exclude possible outliers within the data, the reference values and normalization factors for the minimum requirement to reach a specific content of aroma compounds are derived on the basis of the averaged 10%- or 25%- quantiles, respectively. In the case of commercial NFC-juices, commercial juices from concentrate and restoration aroma water phases, the 10%-quantile was defined as minimal to reach content, for single variety NFC juices the 25%-quantile was taken with regard to the very special composition of some single variety juices and different harvest times. From literature data, where no detailed statistics were given (*6*, *11*), the 10%-quantile was generated by dividing the median or mean value by 5.

The short summary of collected and evaluated data in Table II gives an impression of how the model's reference values were set up. Out of the average content values of the selected compounds in all tested products, average content values in typical apple-juices were determined. For a better application these mean values were rounded to adjusted reference values. Values below 10 were rounded up or down to 5 or 10, respectively, values below 100 were rounded up or down to full ten's and values over 100 to full hundred's.

All ten compounds were weighted equally. No substance class or single compound should be emphasized specifically. The "sum of esters excluding butyl acetate" as proposed by Heil and Ara (*5*) and the "sum of carbon-6-aldehydes and –alcohols" (*12*) were both weighted to 40%. Both were stated to form the basic elements of typical apple-juice aroma (*10*, *15*, *16*). The contents of butyl acetate and 2-methylbutanol were taken into account by 10% each. In order to compensate for the varying content levels of the different compounds and produce a dimensionless index number, weighting factors (f_i) were generated by dividing the weighting (w_i) in % by the reference value (rv_i). $f_i = w_i / rv_i$. For a 10% share of each component the standardization factor was calculated by dividing 10 by the defined adjusted reference value.

The derived standardization factors (Table III) were by and large inversely proportional to the odor threshold values of the aroma compounds in water (cf. Table I). High standardization factors were assigned to compounds with low threshold values, as these compounds contributed more significantly to the general apple juice aroma. Compounds with higher threshold values had a tendency to show minor standardization factors because they needed to be in higher concentration to make a significant contribution to the apple juice aroma. Certainly this tendency is not applicable to some compounds, but overall the comparison of odor threshold values (in water) and the expected contents in apple-juices can clearly be determined.

The aroma index (AI) value is composed of the sum of the products of the content of each aroma compound (A_i) with its standardization factor (f_i).

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch008

In Flavors in Noncarbonated Beverages; Da Costa, N., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

$$\text{Aroma Index (AI)} = A_1*f_1 + A_2*f_2 + \ldots + A_n*f_n$$

To be clearly rated as sufficiently re-aromatized, an Aroma Index of minimally 100 should be attained by an apple juice from concentrate. Failings in the content of certain compounds can be equalized and adjusted for by higher contents of other compounds, while in the model of the sum of esters as proposed by Heil and Ara (*5*), apple varieties with low ester content are generally discriminated. Furthermore the un-weighted model “sum of esters” is mainly shaped by the two quantitatively dominating esters 2-methylbutyl acetate and hexyl acetate, which, because of their comparison to ethyl 2-methylbutyrate and ethyl butyrate higher odor threshold values, make a comparatively low qualitative contribution to the apple-juice aroma. As stated before (*12*), the sum of esters shows no explicit correlation to the sensory impression. By using the aroma index, different types of apple juices can be evaluated without dictating a standardized, by special organoleptic preferences formed aroma profile. The importance of the fruity smelling esters is not placed above other compounds which also decisively contribute to the typical apple juice aroma. The weighting of the compounds supplies an evaluation method appropriate to the average composition of apple juice aroma and taking into consideration miscellaneous aroma substances in equal amounts.

The time the mash stands before pressing influences the aroma index, as carbon-6-compounds are increasingly generated enzymatically during this time (*8*). A high concentration of these carbon-6-compounds is generated immediately after the cell structure is destroyed, which in turn give rise to the typical fresh apple juice aroma. The green note produced by these carbon-6-compounds is essential not only for apple juice but also for the typical apple fruit aroma. On the other hand the sum of esters is influenced by several factors as well. Juices processed from ripe but early harvested apples show a rather low ester content. During ripening, ester concentration increases (*12*, *16*) and in overripe fruit, large concentrations are found.

Application of the Aroma Index Model

The application of the aroma index model to various NFC juices and juices from concentrates analyzed in the year 2008 demonstrated how this model can used to reveal insufficient re-aromatization and to evaluate apple-juice aroma.

Application to NFC Juices

By application to a single variety of juices produced in 2008 it can be demonstrated that the data collected in the year 2007 (*12*), on which the aroma index model is based, is representative of a mean diversity and variability of different apple varieties.

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch008

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch008

Table II. Statistical Database – collected in 2007 - on which the Aroma Index has been developed[a]

Aroma Compound	*10%-quantile NFC Juices*	*10%-quantile Restoration Water phases*	*10%-quantile Juices from Concentrate*	*25%-quantile Single Variety NFC Juices*	*Mean "minimum to reach"- value*	*Adjusted Reference Value*
	n = 91 (*5*, *6*, *12*)	n =174 (*6*, *12*)	n = 190 (*5*, *6*, *12*)	n =172 (*6*, *11*, *12*)		
Ethyl 2-methylbuyrate	9	5	2	6	5	5
Ethyl butyrate	40	21	10	23	21	20
Hexyl acetate	35	24	17	28	25	30
2-Methylbutyl acetate	53	22	32	47	37	40
Butyl acetate	195	47	34	164	97	100
Hexanal	120	31	70	292	127	100
(E)-2-Hexenol	111	119	175	474	232	200
(E)-2-Hexenal	195	220	278	1184	438	400
Hexanol	936	352	879	690	643	600
2-Methylbutanol	368	265	395	243	313	300

[a] All units are µg/L

In 2008 the aroma profiles of eighty-one juices of twenty-eight varieties of apple were analyzed and evaluated (Table IV). The analyzed variety range included "green" varieties like Granny Smith, Gehrers Rambour, and Hilde as well as "yellow" and "red" varieties like Elstar, Shampion, and Remo. Some types have been tested at different harvest times in order to make allowances for the degree of ripeness at different dates of processing. The juices were processed and prepared as described in former studies (*12*). Due to the particular preparation of the juices, which included the mash standing for 15 minutes, in order to imitate the typical parameters of the industrial scale Bucher presses, comparatively high amounts of C6-aldehydes and –alcohols were produced.

The set of statistics corresponded by and large with the data evaluated in 2007 (*12*). All compounds occurred in very broad ranges. The minimum content of the esters was found to be zero, the carbon-6-aldehydes and -alcohols were always generated in certain amounts. Depending on variety and harvest date the content of aroma compounds could rise to several 100 or 1000µg/L. The concentrations of the esters strongly depended upon variety and harvest time. This was verified by the large divergence of median and mean value of these compounds, which was caused by extreme values in some samples. The contents of carbon-6-aldehydes and -alcohols showed a standard distribution with quite corresponding median and mean value. This confirmed the assumption of a variety-independent formation of carbon-6-aldehydes and –alcohols.

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch008

Application to Commercial Apple-Juices from Concentrate

By application of the aroma index model to commercial apple juices from concentrate, the model could be tested with regard to its ability to reliably reveal insufficiently re-aromatized products.

In our laboratory fifty-nine commercial apple juices from concentrate were tested in 2008 (Table V). All of these juices were available on the European market. Various manufacturers, price categories and types of packaging were considered and registered in order to cover a broad range of different products.

The contents of aroma compounds in apple juices from concentrate show a similar distribution to that of the juices analyzed in 2007 (*12*). The 10%-quantile values are close to those values considered in the aroma index model as the lower limit. The fact that apple juices from concentrate contain minor amounts of volatile aroma compounds, probably because of technological losses, has been confirmed. The technologically inevitable losses result from applying explicitly legitimate treatment and working processes like degassing, fining, and hot filling.

The content of aroma compounds varied to quite a wide extent, but mean value and median values showed a good correlation. The highest analyzed aroma index was 926 and the lowest 30. The sum total of esters excluding butyl acetate, showed values between 7 and 785µg/L. The "sum of esters excluding butyl acetate" and aroma index correlated quite well, which was not so surprising as the sum of esters was part of the aroma index by 40%.

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch008

Table III. Aroma Index Model

Aroma Compound	*Weighting [%]*	*Reference Value*[a]	*Standardization Factor*
Ethyl 2-methylbutyrate	10	5	2.000
Ethyl butyrate	10	20	0.500
Hexyl acetate	10	30	0.333
2-Methylbutyl acetate	10	40	0.250
Butyl acetate	10	100	0.100
Hexanal	10	100	0.100
(E)-2-Hexenol	10	200	0.050
(E)-2-Hexenal	10	400	0.025
Hexanol	10	600	0.017
2-Methylbutanol	10	300	0.033
	100		

[a] Units are µg/L

The correlation of the aroma index and the "sum of esters excluding butyl acetate" is graphically presented in Figure 1. A reasonable correlation is disrupted by several outliers which because of high contents of carbon-6-aldehydes and –alcohols show a really high aroma index although their sum of esters is under the proposed lower limit of 150µg/L. The most extreme one shows an aroma index of 490 while just containing 119µg/L of esters. Conversely an apple juice with 257µg/L of esters appears to have a comparatively low aroma index of 173. But the aroma index seems to represent the typical apple juice aroma quite well and to be a good model to adjust the lack of certain compound classes.

If the lowest limit for the "sum of esters" is set at 150µg/L as proposed by Heil and Ara (*5*), twenty-five out of the fifty-nine tested apple juices from concentrate have to be judged as insufficiently re-aromatized. That means a failure rate of 42%. This seems to be a quite considerable share of the current commercial market and does not correspond with the sensorial classification of the tested juices. With only two exceptions all juices with "sum of esters" below 150µg/L, but with an aroma index >100 were by sensory analysis found to be of acceptable quality. The two juices that were sensorially unacceptable displayed a rather flat aroma impression. Twelve of the juices with ester content below 150µg/L were even rated as fruity, aromatic or intensive. With the aroma index lower guideline of 100, seven of the juices clearly had to be rated as incorrectly re-aromatized. These seven juices showed an obvious low content of aroma compounds and were also exposed by their "sum of esters". Other than by the "sum of esters excluding butyl acetate", discriminated juices were classified as sufficiently re-aromatized by the aroma index model, because the low content of esters was adjusted by

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch008

Table IV. Statistical Overview of Single Variety Apple Juices tested in 2008[a]

Aroma Compound	*Mean*	*Median*	*Minimum*	*Maximum*
Ethyl 2-methylbutyrate	31	3	0	447
Ethyl butyrate	139	10	0	2336
Hexyl acetate	280	76	0	2559
2-Methylbutyl acetate	466	30	0	4637
Butyl acetate	520	57	0	6365
Hexanal	1263	1010	165	4264
(E)-2-Hexenol	492	457	30	1670
(E)-2-Hexenal	4124	4116	1210	10165
Hexanol	1925	1414	239	8448
2-Methylbutanol	3086	1530	56	22515
Aroma Index	786	610	194	2653
Sum of Esters (*5*)	961	459	3	5587
Sum of Carbon-6-aldehydes and -alcohols (*12*)	7730	7303	3318	12935

[a] All units are µg/L; the Aroma Index is dimensionless

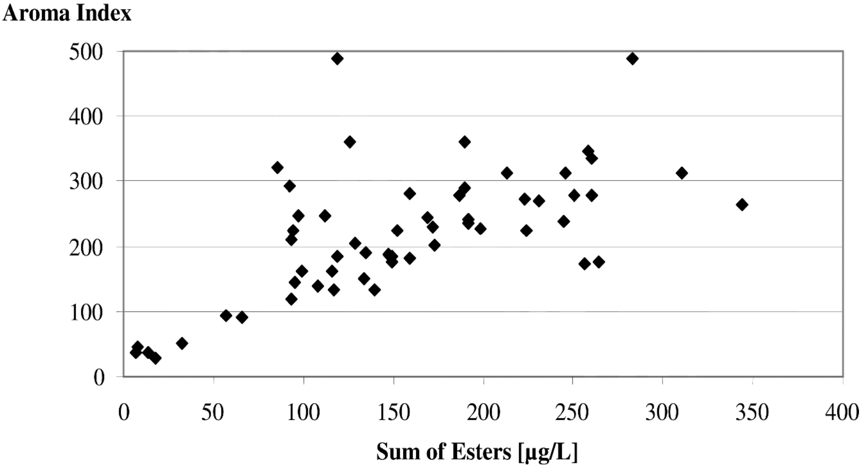


Figure 1. Correlation of Aroma Index and Sum of Esters in Commercial Apple Juices from Concentrate (2008). [1]Two possible statistical anomalies have been omitted from this graph (Sum of Esters/ Aroma Index: 785/926 and 561/540)

higher amounts of other aroma compounds. This corresponded even better with the sensory evaluation of the juices.

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch008

Table V. Statistical Overview of Commercial Apple Juices from Concentrate tested in 2008[a]

Aroma Compound	*Mean*	*Median*	*Min.*	*Max.*	*10%-Quantile*
Ethyl 2-methylbutyrate	18	11	1	119	5
Ethyl butyrate	38	30	2	183	13
Hexyl acetate	37	30	0	163	7
2-Methylbutyl acetate	75	62	0	432	16
Butyl acetate	196	176	1	683	35
Hexanal	114	97	2	356	32
(E)-2-Hexenol	564	473	5	2827	89
(E)-2-Hexenal	693	615	0	2968	132
Hexanol	2296	2000	233	9374	739
2-Methylbutanol	1047	927	158	4591	292
Aroma Index	236	225	30	926	92
Sum of Esters (*5*)	173	150	7	785	64
Sum of Carbon-6-aldehydes and-alcohols (*12*)	3667	2979	423	14673	1451

[a] All units are µg/L; the Aroma Index is dimensionless

Summary

On the basis of analyses carried out in 2007 on various apple juice products, the aroma index model for the evaluation of apple-juice aroma has been developed. Studies executed in 2008 gave proof of the useful application of this model. Sufficiently re-aromatized apple juices can clearly be differentiated from insufficiently re-aromatized ones. With minimal to reach contents as reference values the aroma index was created such that an aroma index of 100 defined the lowest limit for sufficient re-aromatization of apple juice from concentrate. This lowest guideline has been well reasoned and included the whole range of natural variances. Higher values as reference for the compounds included in the aroma index would mean an inappropriate quality standard setting, which could be employed as an internal check, but not set up as law enforced minimum standards. With too high a minimum requirement numerous correctly produced products would unjustifiably be discriminated against.

References

1. EC Council directive 2001/112/EC of December 2001 relating to fruit juices and certain similar products intended for human consumption (ABl. EG 12.01.2002 Nr. L10 S. 58-66).

2. Mosandl, A. Echtheitsbewertung von Fruchtaromen mittels enantioselektiver Kapillargaschromatographie. *Flüssiges Obst.* **1996**, *63*, 386–390.
3. Dietrich, A.; Maas, B.; Karl, V.; Kreis, P.; Lehmann, D.; Weber, B.; Mosandl, A. Stereoisomeric flavor compounds Part LV: Stereodifferentiation of some chiral volatiles on Heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyl-dimethylsilyl)-β-cyclodextrin. *J. High Resolut. Chromatogr.* **1992**, *15*, 176–179.
4. Schurig, V.; Nowotny, H. P. Gaschromatographische Enantiomerentrennung an Cyclodextrinderivaten. *Angew. Chem.* **1990**, *102*, 969–986.
5. Heil, M.; Ara, V. Aroma of fruit juices II – Composition and valuation of apple juice aroma. *Fruit Process.* **2008**, *18* (3), 126–134.
6. Elß, S. *Studien über technologiebedingte Veränderungen der Aromaprofile von Fruchtsäften*, Dissertation, Universität Würzburg, 2007.
7. Schumacher, K.; Asche, S.; Heil, M.; Mittelstädt, F.; Dietrich, H.; Mosandl, A. Methyl-branched flavor compounds in fresh and processed apples. *J. Agric. Food Chem.* **1998**, *46*, 4496–4500.
8. Schieberle, P. Abschlussbericht AiF-FV 13834N. *Einfluss von Sorte und Herstellungsverfahren auf Veränderungen in wertgebenden Aromastoffen von Apfelsäften* **2006**.
9. Quadt, A.; Jülich, E.; Tretzel, J. Sensorische und analytische Beurteilung von Apfelsaftaromen. *Flüssiges Obst.* **2008**, *75* (9), 448–457.
10. Herrmann, K. *Inhaltsstoffe von Obst und Gemüse*; Verlag Eugen Ulmer: 2001.
11. Hey, M.; Kürbel, P.; Hopf, I.; Dietrich, H. Untersuchung sortenreiner Apfelsaftaromen. *Flüssiges Obst.* **2007**, *74* (2), 62–67.
12. Wolter, C.; Gessler, A.; Winterhalter, P. Aspekte zur Beurteilung des Apfelsaftaromas. *Flüssiges Obst.* **2008**, *75* (3), 122−134. English version: Wolter, C.; Gessler, A.; Winterhalter, P. Aspects when evaluating apple-juice aroma. *Fruit Process.* **2008**, 64−80.
13. Arbeitsgruppe "Fruchtsäfte und fruchtsafthaltige Getränke" der Fachgruppe Lebensmittelchemie und gerichtliche Chemie der Gesellschaft Deutscher Chemiker (GDCh), Beurteilung des Saftgehaltes niedersafthaltiger Getränke. 1. Mitteilung: Orangensafthaltige Erfrischungsgetränke. *Flüssiges Obst.* **1979**, *46*, 206; 2. Mitteilung Zitronensafthaltige und grapefruitsaft-haltige Erfrischungsgetränke. *Lebensmittelchemie und gerichtliche Chemie* **1981**, *35*, 79; 3. Mitteilung Apfelsafthaltige Erfrischungsgetränke. *Lebensmittelchemie und gerichtliche Chemie* **1982**, *36*, 60.
14. Belitz, H.-D.; Grosch, W.; Schieberle, P. *Lehrbuch der Lebensmittelchemie*; Springer Verlag, 6. Auflage, 2008.
15. Drawert, F.; Heimann, W.; Emberger, R.; Tressl, R. Über die Biogenese von Aromastoffen bei Pflanzen und Früchten. IV. Mitteilung: Bildung der Aromastoffe des Apfels im Verlauf des Wachstums und bei der Lagerung. *Z. Lebensmittel-Untersuch. u. –Forsch.* **1969**, *140* (2), 65–88.
16. Dimick, P. S.; Hoskin, J. C. Review of apple flavor – State of the art. *Crit. Rev. Food Sci. Nutr.* **1983**, *18* (4), 387–409.

Downloaded by PENNSYLVANIA STATE UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch008

Downloaded by CORNELL UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch009

Chapter 9

Effects of Processing and Storage on the Stability of Folate Vitamers and Pantothenic Acid in Sea Buckthorn Berries and Related Products (*Hippophaë rhamnoides* L. ssp. rhamnoides)

Derek Gutzeit,[1] Gerold Jerz,[1] Michael Rychlik,[2] Bernd Klaubert,[3] and Peter Winterhalter[*,1]

[1]Institute of Food Chemistry, TU Braunschweig, Schleinitzstrasse 20, 38106 Braunschweig, Germany
[2]Chair of Food Chemistry, TU München, Lichtenbergstrasse 4, 85748 Garching, Germany
[3]Bundeswehr Medical Office, Dachauerstrasse 128, 80637 Munich, Germany
[*]p.winterhalter@tu-bs.de

Sea buckthorn has been used for centuries in Eurasia as food (tea, beverages, jam etc.) as well as for ethnomedical remedies. The fruits are known to be a rich source of vitamins, carotenoids, flavonoids, and phytosterols. The awareness of the economic potential of sea buckthorn has resulted in new plantations all over the world. Whereas the high content of vitamin C and carotenoids is well documented, little is known about other water-soluble vitamins. This paper examines the role of *Hippophaë rhamnoides* as a possible source of folate vitamers as well as panthothenic acid.

Introduction

The genus *Hippophaë* (sea buckthorn) is a plant of the family *Elaeagnaceae*, naturally distributed over Asia and Europe. Until now, six species and seven to nine subspecies were established whereas economic relevance is exhibited only by the species *Hippophaë rhamnoides* (*1*). Sea buckthorn is a hardy, deciduous,

In Flavors in Noncarbonated Beverages; Da Costa, N., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

Downloaded by CORNELL UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch009

and dioecious shrub with a height of 2-4m growing preferentially on mountain riversides, and on sandy and gravel grounds. Historically, sea buckthorn was applied as a medicine for horses. The addition of leaves and young branches to the fodder appeared to induce weight gain and to give a shiny coat. Thus, the generic name *Hippophaë* means "shiny (Phae) horse (Hippo)" (*2*). The berries of Hippophaë range in color from yellow to orange-red, in size between 3-8mm in diameter and spherical in shape (*3*). One of the characteristics of sea buckthorn berries is a low pH-value of approximately 2.8 in comparison to other berry fruits. The taste of sea buckthorn berries is generally described as sour and astringent, which is caused by high amounts of organic acids and flavonoids, respectively. The major acid is malic acid with minor amounts of citric and tartaric acids. The sugar/acid ratio and the sweetness in sea buckthorn berries are low in comparison to other berries (*4*). The berries of *Hippophaë rhamnoides* are rich in flavonoids, carotenoids, vitamins, and lipids and are traditionally used for ethnomedicinal remedies in Tibet, Mongolia, China, and Central Asia (*5*). Many health claims are associated with sea buckthorn. In medicinal studies, sea buckthorn berry extracts showed anti-microbial (*6*) and anti-tumoral (*7*) properties. Extracts of *Hippophaë rhamnoides* fruits prevented gastric ulcers in rats (*8*). Beneficial properties of the pulp and seed oil of sea buckthorn in dermatological disorders were reported (*9*). Moreover, vitamin C, tocopherols, tocotrienols, flavonols, carotenoids, and plant sterols have antioxidant capacities and thus they are able to reduce free radical formation (*10–13*). This suggests that *Hippophaë rhamnoides* L. may provide beneficial effects in prevention of coronary heart disease and arteriosclerosis (*14*). Therefore, the high nutritive value of sea buckthorn berries and related products has attracted increasing interest in Europe and North America.

With regard to the volatile constituents of *Hippophaë rhamnoides* only limited data is available. A total of sixty components were identified. The aroma of sea buckthorn fruit was characterized by the presence of several aliphatic esters such as ethyl-, 3-methylbutyl- and *(Z)*-3-hexenyl- esters. The most important compounds were ethyl hexanoate, 3-methylbutyl 3-methylbutyrate, 3-methylbutyric acid, 3-methylbutyl hexanoate, 3-methylbutyl benzoate and 3-methylbutyl octanoate. The concentrations of terpenes and aromatic compounds are low (*15*, *16*). By using malolactic fermentation, it was possible to decrease the acidity and astringency of sea buckthorn juice, while increasing the fruity flavor as well as the fermented flavor (*17*).

This study focused on the investigation of pantothenic acid (vitamin B5) and the group of folate vitamins (vitamin B9) using the stable isotope dilution assay (SIDA), and sensitive detection by electrospray mass-spectrometry (ESI-MS/MS) in sea buckthorn berries, juice and concentrate.

Stable Isotope Dilution Assay

An accurate methodology for the determination and quantification of labile and/or reactive trace compounds in food matrices is the high performance liquid chromatography (HPLC)-technique coupled to mass spectrometry (MS) using

Downloaded by CORNELL UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch009

a stable isotope dilution assay (SIDA) (*18*, *19*). Labeled internal standards ensure that interferences of food matrices have no influence on the results of quantification. Conceivable losses during sample preparation are eliminated by applying SIDA (*20*). By using a labeled standard as the internal standard, which is detectable by its specific mass spectrometric signal in consideration of the mass increment, the analyte can be quantified even more selectively (*21*).

For SIDA four-fold labeled isotopologues of folate vitamins and pantothenic acid were used as internal standards (cf. Figure 1).

Folate

The substance class of folates refers to a group of water-soluble vitamins and represents a class of heterocyclic compounds based on the 4-[(pteridin-6-yl methyl)amino] benzoic acid structure differing by their state of oxidation, their one-carbon substituents, and by the number of attached glutamate residues. In contrast to folic acid, in the natural physiological form of the vitamin the pteridine ring is reduced to give either 7,8-dihydrofolate or 5,6,7,8-tetrahydrofolate. The folate vitamers exist predominantly as polyglutamate derivatives containing up to eight glutamate residues in γ-peptide linkage. A unique structural characteristic of tetrahydrofolate is the stereochemical orientation at the C-6 asymmetric carbon of the pteridine ring. Only the 6*S* stereoisomer is biologically active and synthesized in nature (*22*).

Tetrahydrofolate is extremely sensitive to changing chemical factors such as temperature, oxygen and pH values (*23*–*25*). The oxidation of tetrahydrofolate is described as a free radical chain process (*23*). The reduced folate vitamers act as cofactors in single-carbon transfer reactions in the metabolism of nucleic and amino acids. Various studies over the last decades have reported that folates are supposed to reduce the risk of neural tube defects (*26*), neurological and neuropsychiatric disorders, e.g. Alzheimer disease (*27*) and schizophrenia (*28*), cardiovascular diseases (*29*), and different forms of cancer (*30*, *31*).

The Recommended Dietary Allowance for women and men is set at 400µg/day of dietary folate equivalents. Several studies have shown that fruits and berries are one of the main folate sources providing about 15% of the daily folate intake (*32*, *33*). Moreover, previous investigations analyzed a total folate amount in sea buckthorn berries of about 39µg/100g and 5-methyltetrahydrofolate was characterized as the predominating folate vitamer in berries (*33*).

Pantothenic Acid

Pantothenic acid represents a derivative of pantoic acid connected by a peptide linkage to the amino acid *β*-alanine (*22*). Model experiments have shown that solutions of pantothenic acid are most stable between pH 5.5 and pH 7.0. Below and above these pH values, pantothenic acid is thermolabile and the physiological activity of pantothenic acid (a) is lost by acidic hydrolysis resulting

Downloaded by CORNELL UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch009

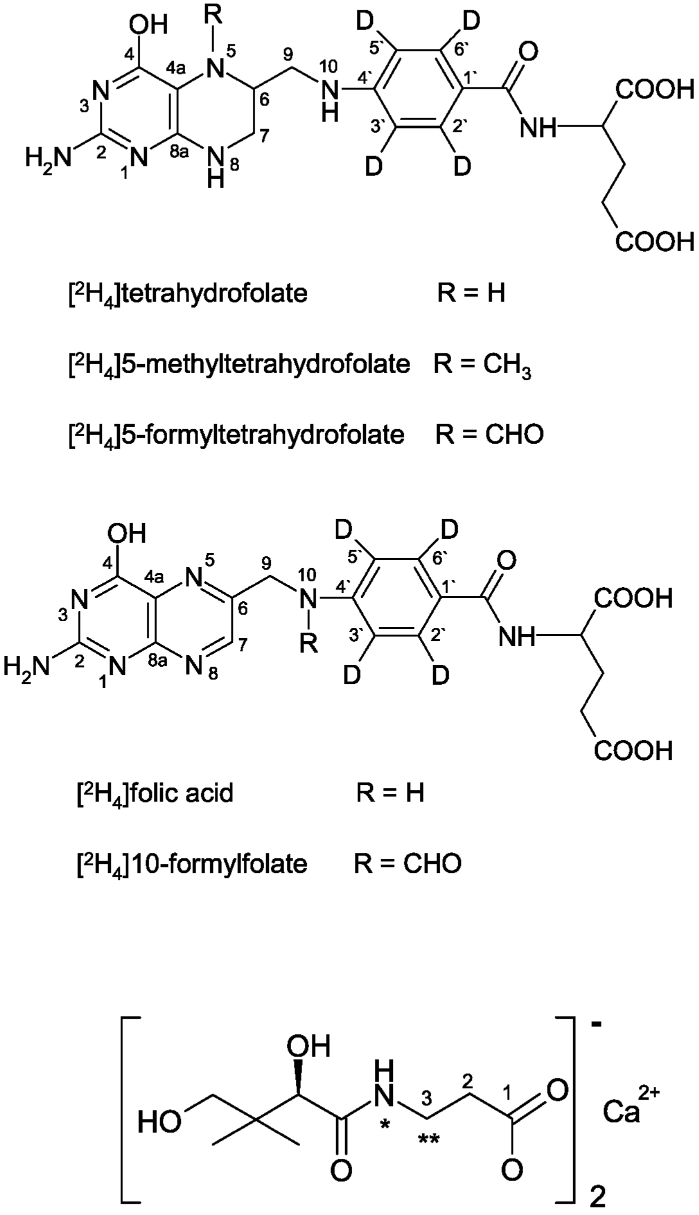

[$^2H_4$]tetrahydrofolate R = H
[$^2H_4$]5-methyltetrahydrofolate R = CH_3
[$^2H_4$]5-formyltetrahydrofolate R = CHO
[$^2H_4$]folic acid R = H
[$^2H_4$]10-formylfolate R = CHO

Figure 1. Structures of the labelled folate vitamers and of calcium [^{15}N, ** $^{13}C_3$]-(R) pantothenate applied to stable isotope dilution assay.*

in two principal cleavage products, namely the γ-lactone (c) of pantoic acid (b) and β-alanine (c) (Figure 2). Pantothenic acid is a precursor of coenzyme A (CoA) and also occurs in phosphopantetheine of the acyl carrier protein (ACP). Hence, coenzymes containing pantothenic acid are involved in several vitally important biological functions, affecting metabolism of carbohydrates, lipids and amino acids. The Recommended Dietary Allowance (RDA) is set at 5mg/d for adults.

Determination of the Folate and Pantothenic Acid Content Using a Stable Isotope Dilution Assay

Folate

Due to their occurrence in trace amounts, their lability, and also the high variety of vitamers, analysis of folates requires several precautions to obtain accurate data. Using stable isotopically labeled analogues of the folate vitamers as internal standards corrects for all losses occurring during the extraction and determination procedure. Enzymatic deconjugation will transfer polyglutamates to their monoglutamic forms and then it is possible to analyze all important vitamers. To enhance sensitivity, the extraction solvent and all subsequent solutions contained ascorbic acid and 2-(mercapto)-ethanol for maximum stability of the folates. Deconjugation by treatment with rat plasma and a preparation of chicken pancreas converted polyglutamic vitamers to the respective monoglutamates, which were then detectable by HPLC/ESI-MS-MS. Subsequently sample clean up was performed using strong anion exchange cartridges, which resulted in the HPLC/ESI-MS-MS chromatograms virtually devoid of matrix interferences as displayed in Figure 3 (*34*).

Pantothenic Acid

Mass spectrometric quantification of pantothenic acid was performed by electrospray ionization (ESI) in positive ionization mode. Direct injection of sea buckthorn berry, juice and concentrate extracts was into the HPLC-ESI-MS-MS system and rapid elution parameters enabled detection in a complex matrix at a retention time of 6.7 minutes. In the full scan spectrum (*m/z* 50-400 amu) pantothenic acid showed an intense base peak for the protonated molecule at *m/z* 220 $[M+H]^+$ (cf. Figure 4a). ESI-MS-MS fragmentation of *m/z* 220 yielded abundant fragment ions at *m/z* 202 $[M-18]^+$, and at *m/z* 184 $[M-36]^+$ by consecutive losses of two water molecules. The likewise intense MS-MS fragment ion signal at *m/z* 90 $[M-130]^+$ is related to the formation of *β*-alanine (Figure 4b). The characteristic MS-MS fragmentation pattern enabled the identification and selective quantification of pantothenic acid in a complex food matrix such as sea buckthorn juice by means of an external calibration curve (ESTD). By using $[^{15}N, ^{13}C_3]$-(*R*)-pantothenic acid as the internal standard, which is detectable by its base signal at *m/z* 224 (Figure 4e), pantothenic acid can be quantified even more selectively. The fragmentation path is corroborated by the cleavage of four-fold labelled pantothenic acid to give the ion-signal at *m/z* 94 corresponding to the $[^{15}N, ^{13}C_3]$-*β*-alanine moiety (Figure 4f).

Downloaded by CORNELL UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch009

Downloaded by CORNELL UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch009

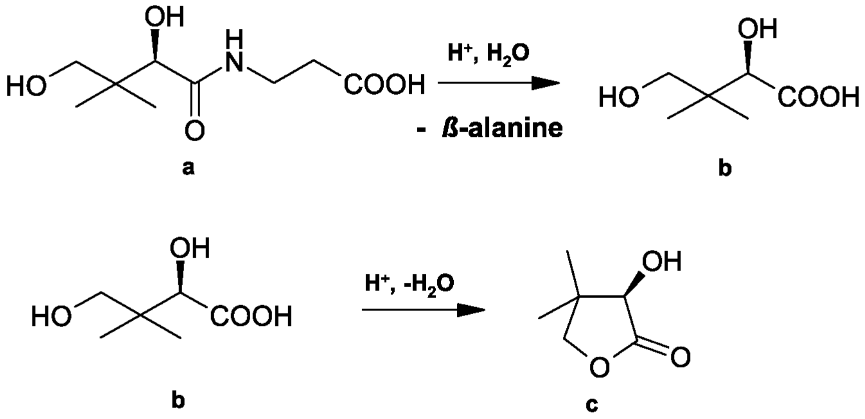


Figure 2. Acidic hydrolysis of pantothenic acid and lactonization of pantoic acid: a: pantothenic acid, b: pantoic acid, c: γ-lactone of pantoic acid.

Effects of Processing on the Stability of Folates and Pantothenic Acid

For our investigations two berry varieties of *Hippophaë rhamnoides* L. ssp. *rhamnoides* were collected. The smaller berry variety was harvested in the southern part of Germany (Area 1) and the second berry variety in Romania (Area 2) from commercial plantings in September 2006.

Folate

Total folate contents of sea buckthorn berries and juices from Area 1 and Area 2 analyzed by SIDA ranged from 29µg/100g up to 81µg/100 g. The major folate derivative in sea buckthorn berries was analyzed as 5-methyltetrahydrofolate with an amount of 31µg/100g (Area 2) and 68µg/100 g (Area 1). The concentration of tetrahydrofolate comprised 14% of the total folate content (berries from Area 2) and was below the limit of detection (< 1.5 µg/100g) in berries from Area 1. Remarkable amounts of 5-formyl tetrahydrofolate of 16µg/100g were exclusively detected in berries from Area 1. The 10-formylfolate and folic acid content in sea buckthorn berries from both growing areas was below the limit of detection (< 0.6µg/100g, < 2.6µg/100g).

5-Methyltetrahydrofolate

The content of 5-methyltetrahydrofolate (31µg/100g) was almost unchanged during the whole processing, from berries to concentrate (Figure 5). This is in contrast to the results of Vahteristo *et al* (*32*), who reported that a juice concentrate prepared from a selection of berries and fruits had a folate content below the detection limit. Obviously, the concentration process during thermovacuum

Downloaded by CORNELL UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch009

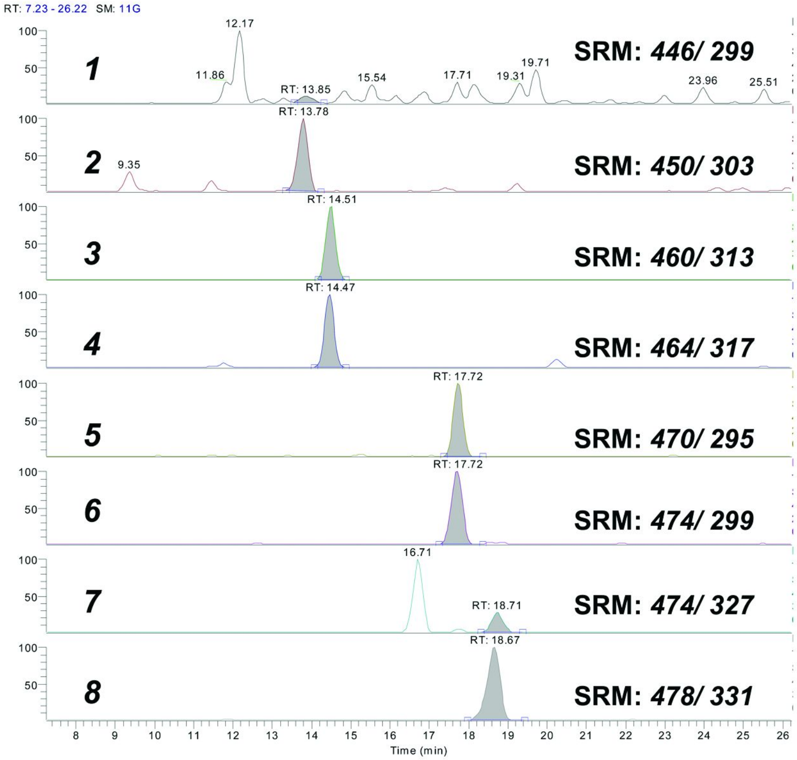


Figure 3. HPLC/ESI-MS-MS chromatogram in positive ionization mode of a folate extract of sea buckthorn juice. Selected reaction monitoring (SRM) traces of folate vitamers and their isotopologues with m/z precursor ion/ m/z product ion: ***1*** *H_4 folate,* ***2*** *[$^2H_4$]-H_4 folate,* ***3*** *5-CH_3 -H_4 folate,* ***4*** *[$^2H_4$]-5-CH_3 -H_4 folate,* ***5*** *10-HCO-folate,* ***6*** *[$^2H_4$]-10-HCO-folate, 7 5-HCO-H_4 folate,* ***8****: [$^2H_4$]-5-HCO-H_4 folate (34).*

evaporation used in our study (five-effect evaporator, 80–85°C) occurred more effectively (loss of approximately 10%).

Tetrahydrofolate and 5-Formyltetrahydrofolate

The juice production process entailed a total degradation of the tetrahydrofolate in sea buckthorn juice (Area 2) (Figure 5). Tetrahydrofolate in particular is extremely sensitive to physical and chemical factors such as temperature, oxygen and pH values (*23–25*). The degradation of the tetrahydrofolate content in sea buckthorn juice (Area 2) was obviously caused by the homogenous distribution of tetrahydrofolate in a liquid matrix and a pH value of 2.8 for sea buckthorn juice compared to other juice matrices. Butz *et al* (*35*) investigated the influence of high-pressure treatment at 25°C and 80°C on folates in orange juice and model media. The experimental data for the model

Downloaded by CORNELL UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch009

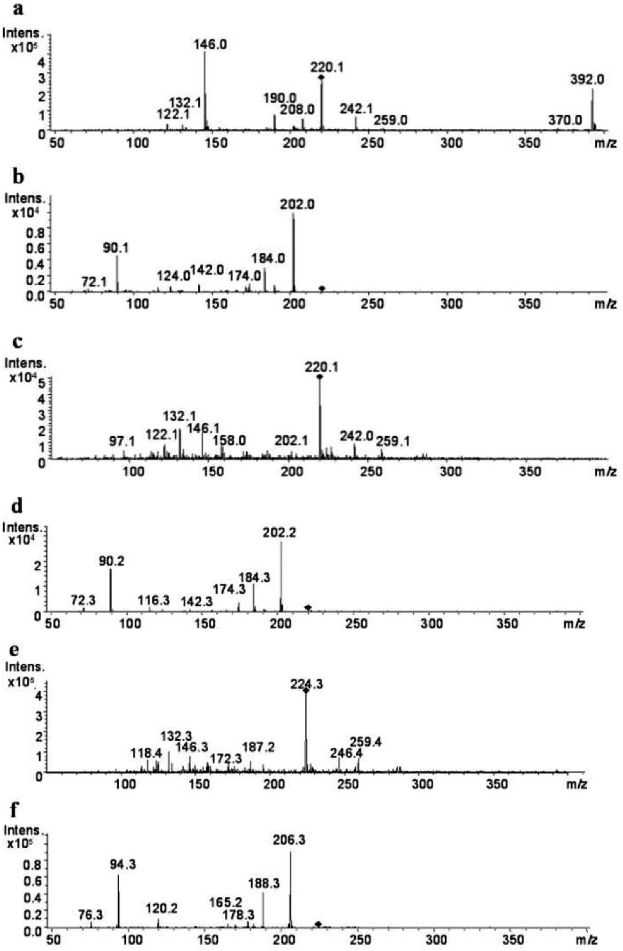


Figure 4. a: Full scan mass spectra of pantothenic acid in sea buckthorn juice, b: MS-MS spectrum (m/z 220) of pantothenic acid in sea buckthorn juice, c: Full scan mass spectra of pantothenic acid standard solution (unlabeled), d: MS-MS spectrum of pantothenic acid standard solution (unlabeled), precursor ion at m/z 220, e: Full scan mass spectra of pantothenic acid standard solution (labelled), f: MS-MS spectrum of [^{15}N, $^{13}C_3$]- pantothenic acid standard solution, precursor ion at m/z 224.

orange juice were well in line with our results. Upon a treatment at 600 MPa and 80°C tetrahydrofolate was almost completely degraded after 6 minutes at a pH of 3.5, without the application of pressure a decay of about 60% after 6 minutes was observed. The investigated sea buckthorn berries (Area 2) exhibited trace amounts (1µg/100g) of 5-formyltetrahydrofolate, which were completely lost during the juice production (Figure 5). Due to the low content of 5-formyltetrahydrofolate in the berries, the latter degradation product was neither detectable in juice nor in the concentrate.

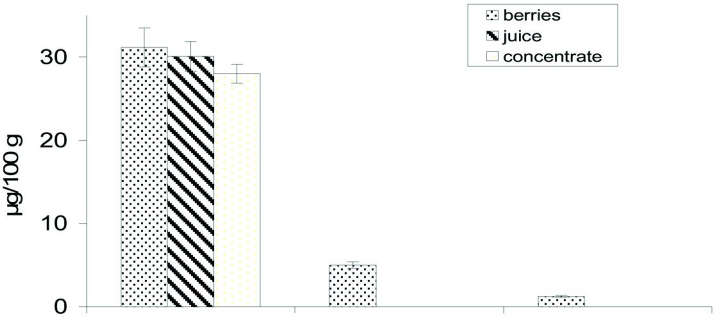


Figure 5. Decrease of 5-methyltetrahydrofolate (5-CH_3-H_4 folate), tetrahydrofolate (H_4 folate) and 5-formyltetrahydrofolate (5-HCO-H_4 folate) of sea buckthorn berries (Area 2) subjected to the commercial manufacturing technique for production of juice and juice concentrate (34).

The production process of sea buckthorn juice and juice concentrate (Area 2) resulted in a degradation of the total folate vitamer contents of 19% and 25%, respectively. In summary it can be ascertained that the processing of sea buckthorn juice and juice concentrate (Area 2) resulted in a lower degradation than expected. Assuming that the average serving size of *Hippophaë* juice in mixtures of fruit juices does not exceed 100g, the folate contribution of sea buckthorn juice (Area 1) may provide an “excellent source” exceeding 20% for achieving the Recommended Dietary Intake for all adults according to the definitions of the U.S. Food and Drug Administration.

Pantothenic Acid

Commercial sea buckthorn juice production includes a high-temperature-short-time treatment (HTST) (90°C, 45 s) before aseptic filling. As the measured pH of the juice was as low as 2.8, a thermal degradation of pantothenic acid in sea buckthorn juice had to be expected. Interestingly, HTST only slightly decreased the pantothenic acid concentration of both areas juices and resulted in a loss of 6.0% in juice of Area 1 and of 6.9% in juice of Area 2 (Table I). During thermovacuum evaporation (five effect evaporator, 80–85°C) of the juice (Area 2), the pH was reduced to 2.6, which was accompanied by a loss of 23.0% in pantothenic acid. However, the production process of sea buckthorn juice resulted in a lower degradation of pantothenic acid than expected. Due to the low processing effects on the pantothenic acid content in juices of both areas, sea buckthorn juice was established as a “good source” exceeding approximately 10% for achieving the Recommended Dietary Allowance for all adults in compliance with the definitions of the U.S. Food and Drug Administration.

Downloaded by CORNELL UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch009

Table I. Pantothenic acid content in sea buckthorn berries, juices, and juice concentrate (*36*)

Food samples		*[mg/kg]*
Hippophaë berries	Area 1	5.46
Hippophaë berries	Area 2	5.09
Hippophaë juice	Area 1	5.13
Hippophaë juice	Area 2	4.74
Hippophaë concentrate (1:6 diluted)	Area 2	3.92

Downloaded by CORNELL UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch009

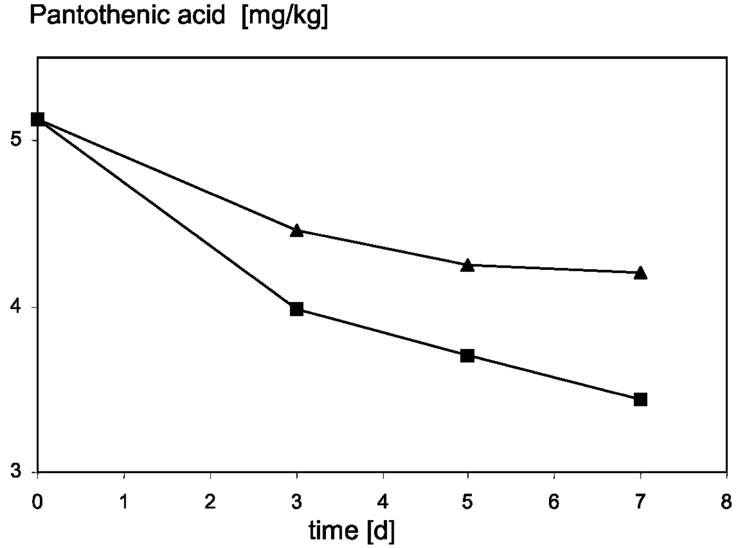


Figure 6. Effect of storage temperature on pantothenic acid degradation in sea buckthorn juice (Area 1). Juice storage at 25 °C (▲) and 40 °C (■) for 7 days (36).

Effects of Storage on the Stability of Folates and Pantothenic Acid

The stability of pantothenic acid in sea buckthorn juice (Area 1) was investigated. Figure 6 shows the effect of storage of sea buckthorn juice at 25°C and at 40°C over seven days. During storage at 25°C for seven days, the pantothenic acid concentration decreased by about 18.1%. In accelerated storage experiments the stability of pantothenic acid was studied at 40°C. After storage for seven days pantothenic acid decreased from 5.13 to 3.44mgkg $^{-1}$, which is equivalent to a loss of 32.9%, thus indicating that pantothenic acid is unstable at elevated temperatures.

Downloaded by CORNELL UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch009

Due to the results of the processing effects the storage investigations were focused on the stability of the 5-methyltetrahydrofolate derivative. The contents of 5-methyltetrahydrofolate and of the total folate esters were approximately unchanged during storage at 6°C after seven days. Almost identical degradation of 5-methyltetrahydrofolate, in the range of about 17-20% at 25°C and 40°C, respectively, was observed for juices after seven days of storage. Interestingly, in comparison to these results the total folate content decreased after seven days at 25°C slowly by 5% and the degradation increased at 40°C up to 17% after seven days. The 17% decline of 5-methyltetrahydrofolate at 25°C and the almost unaffected measured content of total folate under the same storage conditions can be explained by an increase in the 5-formyltetrahydrofolate content of 12µg/100g, which compensates for the loss of 5-methyltetrahydrofolate. These results clearly demonstrated that temperature effects, the most important consumer storage parameters of 6°C and 25°C, do not influence the total folate content of sea buckthorn juice within seven days storage.

Conclusions

The design of juice technological processing parameters has to be optimized to be as gentle as possible to maintain all valuable vitamins such as the amount of pantothenic acid and the group of folates. This means that processing steps have to be monitored by suitable analytical techniques such as mass-spectrometry or the SIDA approach to be able to reduce destructive procedures causing larger losses of unstable vitamins.

We have shown that sea buckthorn juice and its products could be valuable sources of pantothenic acid and folate vitamins, which are known to be extremely important factors in human nutrition.

References

1. Aitzetmüller, K.; Xin, Y. Sanddorn und Sanddornöle – Neuere Entwicklungen in China und Zentralasien. *Nahrung* **1999**, *43*, 228–232.
2. Li, T. S. C. Product development of sea buckthorn. In *Trends in new crops and new uses*; Janick, J.; Whipkey, A., Eds.; ASHS Press: Alexandria, VA, 2002.
3. Li, T. S. C.; Schroeder, W. R. Sea buckthorn (*Hippophae rhamnoides* L.): A multipurpose plant. *HortTechnology* **1996**, *6*, 370–380.
4. Tiitinen, K. M.; Hakala, M. A.; Kallio, H. P. Quality components of sea buckthorn (*Hippophaë rhamnoides*) varieties. *J. Agric. Food Chem.* **2005**, *53*, 1692–1699.
5. Guliyev, V. B.; Gul, M.; Yildirim, A. *Hippophae rhamnoides* L.: chromatographic methods to determine chemical composition, use in traditional medicine and pharmacological effects. *J. Chromatogr., B* **2004**, *812*, 291–307.
6. Puupponen-Pimiä, R.; Nohynek, L.; Meier, C.; Kähkönen, M.; Heinonen, M.; Hopia, A.; Oksmanen-Caldentey, K. M. Antimicrobial properties of phenolic compounds from berries. *J. Appl. Microbiol.* **2001**, *90*, 494–507.

Downloaded by CORNELL UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch009

7. Teng, B. S.; Lu, Y. H.; Wang, Z.; Tao, X. Y.; Wei, D. Z. *In vitro* anti-tumor activity of isorhamnetin isolated from *Hippophae rhamnoides* L. against BEL-7402 cells. *Pharmacol. Res.* **2006**, *54*, 186–194.
8. Süleyman, H.; Demirezer, L. Ö.; Büyükokuroglu, M. E.; Akcay, M. F.; Gepdiremen, A.; Banoglu, Z. N.; Göçer, F. Antiulcerogenic effect of *Hippophae rhamnoides* L. *Phytother. Res.* **2001**, *15*, 625–627.
9. Yang, B.; Kalimo, K. O.; Mattila, L. M.; Kallio, S. E.; Katajisto, J. K.; Peltola, O. J.; Kallio, H. P. Effects of dietary supplementation with sea buckthorn (*Hippophaë rhamnoides*) seed and pulp oils on atopic dermatitis. *J. Nutr. Biochem.* **1999**, *10*, 622–630.
10. Geetha, S.; Sai Ram, M.; Singh, V.; Ilavazhagan, G.; Sawhney, R. C. Antioxidant and immunomodulatory properties of seabuckthorn (*Hippophae rhamnoides* L.) – an *in vitro* study. *J. Ethnopharmacol.* **2002**, *79*, 373–378.
11. Kallio, H.; Yang, B.; Peippo, P.; Tahvonen, R.; Pan, R. Triacylglycerols, glycerophospholipids, tocopherols, and tocotrienols in berries and seeds of two subspecies (ssp. *sinensis* and *mongolica*) of sea buckthorn (*Hippophaë rhamnoides*). *J. Agric. Food Chem.* **2002**, *50*, 3004–3009.
12. Kallio, H.; Yang, B.; Peippo, P. Effects of different origins and harvesting time on vitamin C, tocopherols, and tocotrienols in sea buckthorn (*Hippophaë rhamnoides*) berries. *J. Agric. Food Chem.* **2002**, *50*, 6136–6142.
13. Rösch, D.; Bergmann, M.; Knorr, D.; Kroh, L. W. Structure-antioxidant efficiency relationships of phenolic compounds and their contribution to the antioxidant activity of sea buckthorn juice. *J. Agric. Food Chem.* **2003**, *51*, 4233–4239.
14. Zeb, A. Important therapeutic uses of sea buckthorn (*Hippophae*): A review. *J. Biol. Sci.* **2004**, *4*, 687–693.
15. Hirvi, T.; Honkanen, E. The aroma of the fruit of sea Buckthorn, *Hippophae rhamnoides* L. *Z. Lebensm. Unters. Forsch.* **1984**, *179*, 387–388.
16. Tiitinen, K.; Hakala, M.; Kallio, H. Headspace volatiles from frozen berries of sea buckthorn (*Hippophaë rhamnoides* L.) varieties. *Eur. Food Res. Technol.* **2006**, *223*, 455–460.
17. Tiitinen, K.; Vahvaselkä, M.; Laakso, S.; Kallio, H. Malolactic fermentation in four varieties of sea buckthorn (*Hippophaë rhamnoides* L.). *Eur. Food Res. Technol.* **2007**, *224*, 725–732.
18. Jezussek, M.; Schieberle, P. A new LC/MS-Method for the quantitation of acrylamide based on a stable isotope dilution assay and derivatization with 2-mercaptobenzoic acid. Comparison with two GC/MS methods. *J. Agric. Food Chem.* **2003**, *51*, 7866–7871.
19. Rychlik, M. Simultaneous analysis of folic acid and pantothenic acid in foods enriched with vitamins by stable isotope dilution assays. *Anal. Chim. Acta* **2003**, *495*, 133–141.
20. Freisleben, A.; Schieberle, P.; Rychlik, M. Specific and sensitive quantification of folate vitamers in foods by stable isotope dilution assays using high-performance liquid chromatography-tandem mass spectrometry. *Anal. Bioanal. Chem.* **2003**, *376*, 149–156.
21. Rychlik, M.; Asam, S. Stable isotope dilution assays in mycotoxin analysis. *Anal. Bioanal. Chem.* **2008**, *390*, 617–628.

Downloaded by CORNELL UNIV on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch009

22. Ball, G. F. M. *Vitamins in foods. Analysis, bioavailability, and stability*; CRC Press Taylor & Francis Group: Boca Raton, FL, 2006.
23. Pearson, A. J. Kinetics and mechanism of the autoxidation of tetrahydropterins. *Chem. Ind.* **1974**, *6*, 233–239.
24. Reed, S.; Archer, M. C. Oxidation of tetrahydrofolic acid by air. *J. Agric. Food Chem.* **1980**, *28*, 801–805.
25. Gregory, J. F., III. Chemical changes in vitamins during food processing. In: *Chemical changes of food during processing*; Richardson, T., Finley, J. W., Eds.; Van Nostrand Co.: New York, 1985; pp 373−408.
26. Wald, N.; Sneddon, J.; Densem, J.; Frost, C.; Stone, R. Prevention of neural tube defects: Results of the Medical Research Council Vitamin Study. *Lancet* **1991**, *338*, 131–137.
27. Snowdon, D. A.; Tully, C. L.; Smith, C. D.; Riley, K. P.; Markesbery, W. R. Serum folate and the severity of atrophy of the neocortex in Alzheimer disease: findings from the Nun Study. *Am. J. Clin. Nutr.* **2000**, *71*, 993–998.
28. Regland, B. Schizophrenia and single-carbon metabolism. *Prog. Neuro-Psychopharmacol. Biol. Psych.* **2005**, *29*, 1124–1132.
29. Robinson, K.; Homocysteine, B vitamins, and risk of cardiovascular disease. *Heart* **2000**, *83*, 127–130.
30. Zhang, S.; Hunter, D. J.; Hankinson, S. E.; Giovannuci, E. L.; Rosner, B. A.; Colditz, G. A.; Speizer, F. E.; Willett, W. C. A prospective study of folate intake and the risk of breast cancer. *J. Am. Med. Assoc.* **1999**, *281*, 1632–1637.
31. Kim, Y. I. Role of folate in colon cancer development and progression. *J. Nutr.* **2003**, *133*, 3731S–3739S.
32. Vahteristo, L.; Lehikoinen, K.; Ollilainen, V.; Varo, P. Application of an HPLC assay for the determination of folate derivatives in some vegetables, fruits and berries consumed in Finland. *Food Chem.* **1997**, *59*, 589–597.
33. Strålsjö, L.; Åhlin, H.; Witthöft, C. M.; Jastrebova, J. Folate determination in Swedish berries by radioprotein-binding assay (RPBA) and high performance liquid chromatography (HPLC). *Eur. Food Res. Technol.* **2003**, *216*, 264–269.
34. Gutzeit, D.; Mönch, S.; Jerz, G.; Winterhalter, P.; Rychlik, M. Folate content in sea buckthorn berries and related products (*Hippophaë rhamnoides* L. ssp. *rhamnoides*): LC-MS/MS determination of folate vitamer stability influenced by processing and storage assessed by stable isotope dilution assay. *Anal. Bioanal. Chem.* **2008**, *391*, 211–219.
35. Butz, P.; Serfert, Y.; Garcia, A.; Fernandez; Dieterich, S.; Lindauer, R.; Bognar, A.; Tauscher, B. Influence of high-pressure treatment at 25°C and 80°C on folates in orange juice and model media. *J. Food Sci.* **2004**, *69*, SNQ117–SNQ121.
36. Gutzeit, D.; Klaubert, B.; Rychlik, M.; Winterhalter, P.; Jerz, G. Effects of Processing and of Storage on the Stability of Pantothenic Acid in Sea Buckthorn Products (*Hippophae rhamnoides* L. ssp. *rhamnoides*) Assessed by Stable Isotope Dilution Assay. *J. Agric. Food Chem.* **2007**, *55*, 3978–3984.

Downloaded by UNIV OF DELAWARE MORRIS LIB on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch010

Chapter 10

Processing Effects on the Flavor and Quality of Blueberries

Lisa L. Dean,[1,*] Je'Velle B. Leavens,[2] and Leon C. Boyd[2]

[1]Market Quality and Handling Research Unit, USDA, ARS, Raleigh, NC 27695-7624, USA
[2]Deparment of Food, Bioprocessing, and Nutritional Sciences, North Carolina State University, Raleigh, NC 27695-7624, USA
[*]Lisa.Dean@ars.usda.gov

The valuable nutrients and antioxidants present in fruits and berries are responsible for their perception as healthy foods. To prepare these products for juices and wines, they are often heated and pasteurized. This can cause flavor changes and losses of antioxidant compounds in blueberries. Changes induced by traditional hot pasteurization were compared to newer processing technologies such as enzyme treatment and high hydrostatic pressure. Consumer perceptions of the resulting products and changes in the flavor quality and the antioxidant retention showed that degradation was not lessened by the newer processes.

Introduction

Fruit and vegetable consumption in the United States varies among the population for a number of reasons. It is subject to the economic status of the consumer, in that low income households or individuals may not have access to supply due to the lack of supermarkets in inner city and rural areas (*1*). Retail prices of fresh produce have traditionally increased twice as much as all other foods over the past twenty years. This also has a bearing on the affordability of these items to certain portions of the population (*2*).

Actual and potential health benefits also play a part in that studies have shown that diets high in fruits and vegetables are associated with lower incidences of

In Flavors in Noncarbonated Beverages; Da Costa, N., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

chronic diseases such as heart disease, certain cancers, and metabolic syndromes (*3–6*). Thus consumption of these products is to be encouraged. Fruits and the byproducts of their processing are known sources of vitamins, minerals, and antioxidant compounds. The Center for Disease Control's (CDC) "Five a Day" program encourages everyone to consume five servings of fruits or vegetables each day to protect against chronic diseases, help with weight management, calorie balance, energy, and to maintain good general health (*7*).

It is often necessary to process fruits and vegetables thermally to preserve them or to make them more palatable, but this often results in a number of changes (*8*). These processes include the release of compounds with potential antioxidant properties from complexes with sugars and proteins. Oxidases and other enzymes are inactivated resulting in less deterioration. There are also negative effects such as changes in color, flavors and loss of nutrients such as vitamins.

Downloaded by UNIV OF DELAWARE MORRIS LIB on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch010

Juice Production from Fruits

Technologies that have been developed for processing berries and small fruits often involve the productions of juices. Typically, for commercial sale, juice is expressed from the fruit by mechanical pressure and then the resulting juice is cleared of haze by filtration or by the use of clarifying agents. The juice is then heat pasteurized and bottled. Newer technologies have been developed that show promise for producing high quality juices with good flavor retention. These include hot pressing, use of high hydrostatic pressure for juice extraction and enzyme treatments for juice release.

Conventional Juice Processing

Fruit juice products available for retail sale are often produced by pressing fruits, collecting the juices and concentrating them. In the interest of economy, this allows them to be transported more economically by reducing the water content. Many products are also blends of less costly juices such as apple, pear and white grape that have very bland flavors, but still allow the label to state "100%" juice (*9*). These products are often flavored with natural and artificial flavors as well. Vitamins and minerals can be added to fortify the final product so that it is able to compete nutritionally with single juice products.

Hot Pressing

In some cases, juices can be produced by hot pressing fruits. In this case, the fruit is heated under steam to temperatures up to 75°C, crushed and then rapidly cooled. The juice is then strained to remove seeds and pulp and then pasteurized for packaging. This type of pressing has been found in some cases to increase juice yields, to give more appealing colors with fruits such as muscadine grapes, as well as more acceptable flavors (*10*).

High Hydrostatic Pressure Processing

This type of processing has been studied for several decades. It is considered an ideal way to produce microbiologically safe products without cooking, which can destroy colors and flavors. In this unit operation, food products are usually packaged into a flexible packaging material and then subjected to high hydrostatic pressures of 400 to 600MPa with or without heating (*11*). Originally foods such as oysters and clams were treated so that they would be free of microbial contamination, but still maintain their "raw" characteristics (*12*). There are reports in the literature about juices being further processed to reduce the microbial load in the juice (*13*, *14*), thus it still appears to be an emerging technique for the processing of fruit juices. The advantages of these systems is that the flavors and the nutritionally active compounds such as vitamins are less impacted than by the use of conventional heat process techniques (*15*, *16*). As this technique becomes more prevalent, it would be expected that more juice products will be processed using it.

Downloaded by UNIV OF DELAWARE MORRIS LIB on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch010

Enzymatic Release

The use of enzymes such as pectinases to aid in the release of juice from fruit pulp is a technology in common use (*17–19*). It has been found that this technique increases the yield of juice from fruits and vegetables over that of simple mechanical pressing. Treatment with pectinases also aids in clarification of juices by reducing turbidity due to the colloidal substances still present after pressing (*20*). This treatment has been found to be effective in reducing juice viscosity, total pulp and to increase the production of juice without changing the pH or the levels of total acidity, vitamin C or total solids (*21*). The wine industry has also made use of this technique and there exists specific enzyme products that are marketed for the treatment of wine grapes. The appeal of the use of these products is that certain glycosides are broken down which release aroma compounds that add to the flavor experience (*22*). It must be noted that specific enzymes used will affect the juice yield, but not necessarily the anthocyanin content of the resulting juice. This seems to be dependent on the pectin depolymerization activity (*23*).

North Carolina is one of the leading producers of blueberries. Over the period of 2006 to 2008, North Carolina was the fourth ranking state in the US (*24*). It is therefore important to find novel processing technologies or modified techniques that can be utilized to increase the production and optimization of "value-added" blueberry products, thus increasing the economic impact of the North Carolina blueberry industry. To meet consumers' demands for products with bioactive components rich in antioxidants, it is essential for food processors to know the affect of processing procedures on the nutrient concentration and the bioactivity of compounds present in the final product. The objective of this study was to determine the effects of different processing techniques including thermal processing and high hydrostatic pressure (HHP) on the antioxidant activity of blueberry juice. Antioxidant levels were measured by changes in total anthocyanins, total phenols, and Oxygen Radical Absorbance Capacity (ORAC) values. Consumer acceptability of the juice products produced under some of the different treatments was also determined by means of sensory panels.

Materials and Methods

Sample Juice Preparation

Samples included juices prepared from individually quick frozen (IQF) Croatan blueberries using thermal processing, cold processing (22°C) and hot processing (43°C and 75°C) for 30 sec in a steam jacketed kettle before pressing. Samples were pasteurized by heating for 2 sec at 160°C using steam and an in house built parallel plate device. A blueberry juice blend was prepared with pure apple juice using frozen pasteurized concentrate in order to modify the strong flavor of pure blueberry juice. A commercial product labeled as 100% blueberry juice was purchased from a local grocery store to be used as a comparison sample. The commercial sample was blended with the same apple juice to produce a product containing 80:20 blueberry: apple juice (vivo). The blended juices were prepared no more than 24 hr before sensory evaluations. To prepare the sensory portions, 30mL of juice was placed into 4oz clear plastic cups labeled with random three digit codes, capped and placed in a refrigerator until presented to the panelists for evaluation. A total of four samples were presented for consumer evaluation.

Sensory Evaluation Procedure

After approval by the Institutional Review Board at North Carolina State University, seventy-nine panelists were recruited from the university community via posted fliers and electronic mail. All subjects signed consent forms and filled in a consumer questionnaire regarding juice purchases before testing. The four samples were presented at one time, in random order, and each panelist was asked to taste the samples in the order presented. Panelists were given water to clear the palate between samples.

The questionnaire was planned so that panelist rated each sample using a nine-point hedonic scale with nine structural levels from 1 “dislike extremely” through 5 “neither like nor dislike” to 9 “like extremely” for overall acceptability, blueberry flavor, sweetness, overall flavor, overall thickness or mouthfeel and overall appearance liking. The intensity levels for blueberry flavor and sweetness were evaluated by the panelists from 1 “low” through 5 “moderate” to 9 “high”. The panelists evaluated each sample for overall acceptability, flavor, sweetness, mouthfeel, and appearance.

Downloaded by UNIV OF DELAWARE MORRIS LIB on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch010

Downloaded by UNIV OF DELAWARE MORRIS LIB on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch010

Determination of Antioxidant Activity

There are many tests that can be used to measure the activity of compounds present in fruit and vegetables against oxidative attack. Here we present the results from our analyses using some of the more common methods. These were chosen so that the results could be more readily compared to those in the literature.

Total Anthocyanins and Polymetric Color

The pH differential method was used to determine the monomeric anthocyanin content of the juice samples (*25*). In brief, juice samples were diluted using 0.025mol/L potassium chloride buffer (pH 1.0) and 0.4mol/L sodium acetate buffer (pH 4.5). The absorbance was measured at 520 and 700 nm after 15 min incubation using a spectrophotometer (Spectronic Gynesis 2 UV-Vis, Thermo Electron Corp., Madison, WI). The calculation can be found in the reference (*26*).

The juice samples were also analyzed for their percent colorimetric content using the bisulfite bleaching method described in Guisti and Worlstad (*26*). After dilution with either potassium metabisulfite solution or distilled water, the juices were held at room temperature for 15 min. The absorbance of the solutions was read at 420, 520 and 700nm. Using the equations described in the reference, the color density, polymeric color and % polymeric color were determined.

Total Phenolics

The Folin-Ciocalteu method was used to determine the total phenolic content of the juices as gallic acid equivalents (*27*). In brief, juice samples were mixed with diluted Folin-Cioalteu reagent. After 5 min, sodium carbonate solution was added and the mixture was incubated for 90 min at 22°C. The absorbance was read at 725nm and the total phenolic content was determined by comparison against a standard curve of gallic acid.

Oxygen Radical Absorbance Capacity (ORAC)

The ability of the juices to inhibit or decrease the degradation of fluoroscein (Sigma Chemical Corp., St. Louis, MO) in the presence of 2,2’-azobis(2-amidino-propane dihydrochloride) (Wako Chemicals USA, Inc., Richmond, VA) was determined according to the method of Prior, *et al* (*28*). This was done using a Tecan Safire microplate reader (Tecan US, Durham, NC). A standard curve was prepared using Trolox, a water soluble Vitamin E analogue (MP Biomedicals, Inc., Salon, OH) to quantify the antioxidant activity using the regression equation (y=a+bx) between the Trolox concentration and the net area under the fluorescence curve. The ORAC value was expressed as μmoles Trolox equivalents/L. (TE/L).

Downloaded by UNIV OF DELAWARE MORRIS LIB on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch010

Physiochemical Analyses

The total soluble solids (TTS) were determined as degrees Brix using a hand-held refractometer (Chase Instrument Corp., Amityville, NY). Titratable acidity as citric acid was determined using AOAC method 950.21 (*25*). In brief, 10mL of juice was diluted to 100mL and titrated with 0.1mol/L sodium hydroxide to an end point of pH 8.2.

Statistical Analysis

The data were analyzed using ANOVA to determine differences between the means of the treatments. Fisher's Least Significant Difference ($p<0.05$) was used to separate the means using SAS 8.2 (Cary, NC).

High Hydrostatic Pressure Processing

Additional trials were conducted by subjecting the IQF blueberries to enzyme treatment and subsequent high pressure treatment. For this, 4.5kg of berries were pulverized using a hand-operated pulper. The resulting crushed berries were treated with the pectinase, Rapidase (Presque Isle Wine Cellars, North East, PA). The enzyme was added at a rate of 45mL/g crushed weight and subsequently the berries were macerated and chilled overnight at 3°C. The berry slurry samples were sealed into plastic pouches with a Model H-306 heat sealer (U Line Industries, Chicago, IL). Each pouch held approximately 300g of material. The pouches were then subjected to pressure at 400MPa at 10, 20 and 30 min holding times using a CIP 22260 Pressure Unit (Autoclave Engineers, Erie, PA). The solid material was removed by passing the slurry through a stainless steel basket bladder press with 100% cotton pressing cheesecloth (Presque Isle Wine Cellars, North East, PA).

Results and Discussion

Effects of Thermal Processing on Bioactivity of Blueberry Juice

Using the initial weight of the crushed berries, the juice yields were calculated for each processing treatment. They were found to increase with increasing temperature of processing. The hot processing at 75°C resulted in a yield of 87.8% compared to 66.1% and 84.6% at 22°C and 43°C, respectively. The yield of total phenolics followed a similar pattern to that of the juice yield in that as the temperature increased, the total phenolic compounds extracted increased (Figure 1). Along with the increase in phenolics released possibly due to increased cell permeability, the total antioxidant levels as measured by ORAC were found to be increased as well (Figure 2). This is as to be expected since the ORAC measures

Downloaded by UNIV OF DELAWARE MORRIS LIB on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch010

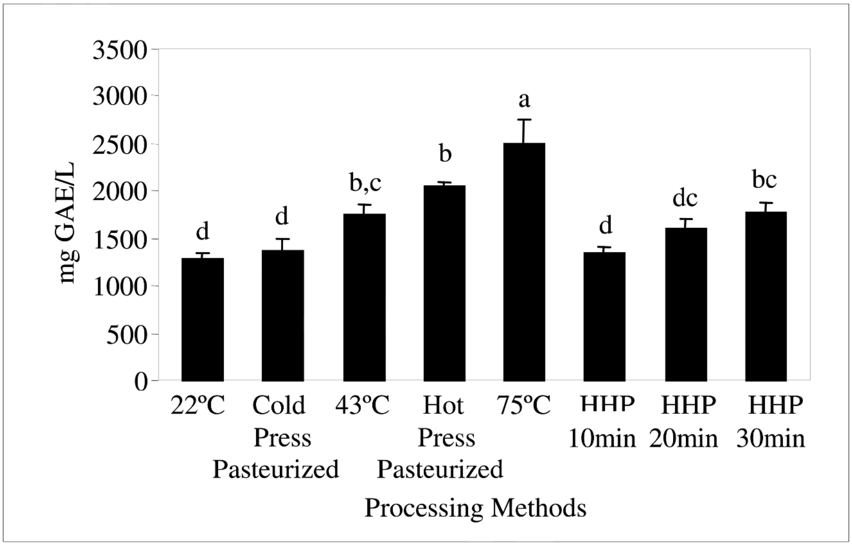


Figure 1. Total phenolics in gallic acid equivalents per L of juice (mg GAE/L) measured in blueberry juices after heat treatments and high pressure processing (HHP). Significant differences between treatments are indicated by different letters ($p<0.05$).

the ability of compounds present to transfer hydrogen's to free radicals involving active oxygen (*29*). Since phenolics are very proficient at this, any increase in phenolic content would correspond to an increase in ORAC value as well. The USDA ORAC table's list values for a wide range of food materials and our results were found to be comparable and show that blueberry juice would be a source of compounds with significant ORAC activity (*30*).

Effects of Thermal Processing on Blueberry Juice Color and Flavor

The thermal processes also resulted in increases in the anthocyanin content which would correspond to increasing darker colors of the juice. This was supported by higher numbers in the color density index (data not shown) with the higher temperature processing. The release of these compounds during juice processing would be expected to be reflected in the flavor. Certain small molecule phenolics have been associated with astringent and bitter flavors (*31*, *32*). The physiochemical characteristics of the blueberry juice blends prepared in the laboratory were found to be very similar to a commercial product (Table I).

Consumer evaluation of the juices prepared by the various processing treatments and the commercial juice showed that the panelists found significant differences between the samples in most cases as seen in Table II. In most cases, the cold pressed juice and the commercial juice were scored lower in liking and acceptability than the hot pressed products. This was especially evident in the

Downloaded by UNIV OF DELAWARE MORRIS LIB on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch010

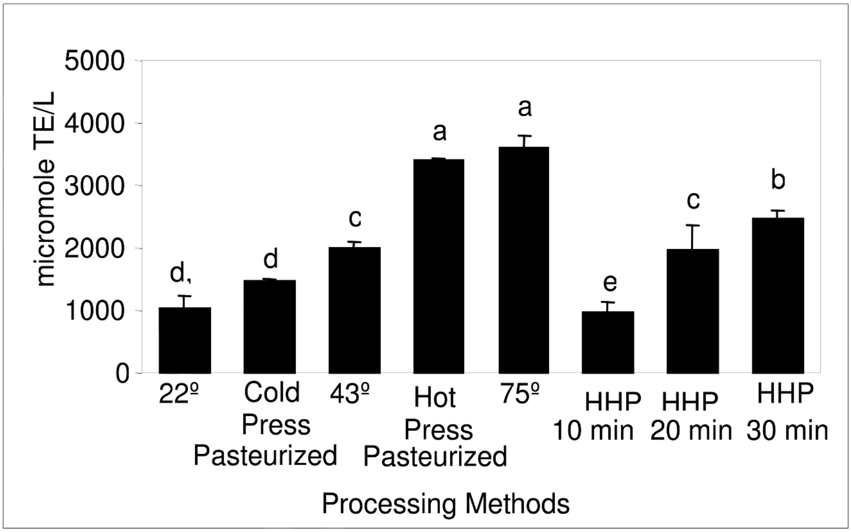


Figure 2. Total Oxygen Radical Absorbance Capacity (ORAC) as micromoles Trolox Equivalents (TE) per L of juice in blueberry juices after heat treatments and high pressure processing (HHP). Significant differences between treatments are indicated by different letters (p<0.05).

Table I. Physiochemical Characteristics of Blueberry Juice Blends

Characteristic	*CP*[1]	*HP_1*[1]	*HP_2*[1]	*Commercial Juice*
pH	3.47[1]	3.48	3.49	3.32
Titratable Acidity (%)	0.36	0.43	0.44	0.39
°Brix	13.1	13.0	13.1	14.0

[1] CP, HP_1, HP_2 represent the pasteurized juices prepared from blueberries subjected to cold press, hot press 43°C, and hot press 75°C processing methods respectively.

overall appearance and maybe related to the stability of natural fruit anthocyanins. The freshly processed juices with their higher color values may have had more appeal to the test panelists. The addition of heat has been noted to increase the development and release of volatile flavor compounds in the berry skins, which in this case may have been responsible for the perceived increase in the flavor intensity of the hot processed juice samples (*33*).

Consumer Choices in Selecting Juice Products

As part of the consumer panel, participants were asked what market influences affected their choice of juice beverages. The ballot asked each participant to rank appearance, availability, price, perception of health effects and flavor. It was found that the highest consumer rankings of these choices were price, flavor, and health

Downloaded by UNIV OF DELAWARE MORRIS LIB on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch010

Table II. Mean Hedonic Rating from Consumer Acceptability Test

Sensory Attributes	*CP*^{1}	*HP*$_{1}^{1}$	*HP*$_{2}^{1}$	*Commercial Juice*
Overall Acceptability	4.78^{c}	6.05^{a}	6.33^{a}	5.30^{b}
Berry Flavor Intensity	5.13^{b}	6.32^{a}	6.33^{a}	5.07^{b}
Berry Flavor Liking	4.56^{c}	6.03^{a}	6.07^{a}	5.30^{b}
Sweetness Intensity	5.06^{b}	5.60^{a}	5.77^{a}	5.53^{a}
Sweetness Liking	5.14^{c}	5.72^{b}	6.35^{a}	5.52^{bc}
Overall Flavor Liking	4.51^{c}	5.93^{a}	6.32^{a}	5.23^{b}
Overall Mouthfeel	5.25^{b}	6.39^{a}	6.43^{a}	5.68^{b}
Overall Appearance	5.70^{b}	6.94^{a}	7.16^{a}	4.14^{c}

[1] CP, HP_1, HP_2 represent the pasteurized juices prepared from blueberries subjected to cold press, hot press 43°C, and hot press 75°C processing methods respectively. [a,b,c] Statistical analysis ANOVA and Fisher's LSD were performed using SAS 8.2. Mean values in a row followed by different letters are significantly different ($p<0.05$).

respectively. This indicates that even though appearance would logically seem to be an important parameter in selecting a juice product, consumers rank flavor as more important. Maintenance of high quality flavor by use of optimum processing techniques for preservation and safety is critical.

Effects of High Hydrostatic Pressure (HHP) Treatment on Blueberry Juice Quality

The high pressure treatments resulted in higher recoveries of the anthocyanins with time, but these did not reach the levels accomplished by the hot pressed pasteurized samples alone (Figure 3). The same trend was seen with the phenolics and the ORAC activity (Figures 1, 2). This indicates that while such processing would be effective for reducing the microbial content of fruit juices, it would maintain color and bioactivity. This is in agreement with the findings of Suthanthangjai *et al* (*34*) in regards to raspberries. They found that the use of HHP in addition to refrigerated storage allowed for the retention of more anthocyanins. Samples were found to have lost only 12 to 14% of their total anthocyanin content as compared to 63 to 70% when the juice was stored at 30°C.

Since we were unable to develop a process using HHP that was equivalent to pasteurization and insure the safety of the juices, we did not conduct consumer panels with the juices treated with HHP. The lower results for the total phenolic values (Figure 3) would indicate that these samples could be less bitter than the hot pressed pasteurized juices tested and might have similar taste appeal.

Downloaded by UNIV OF DELAWARE MORRIS LIB on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch010

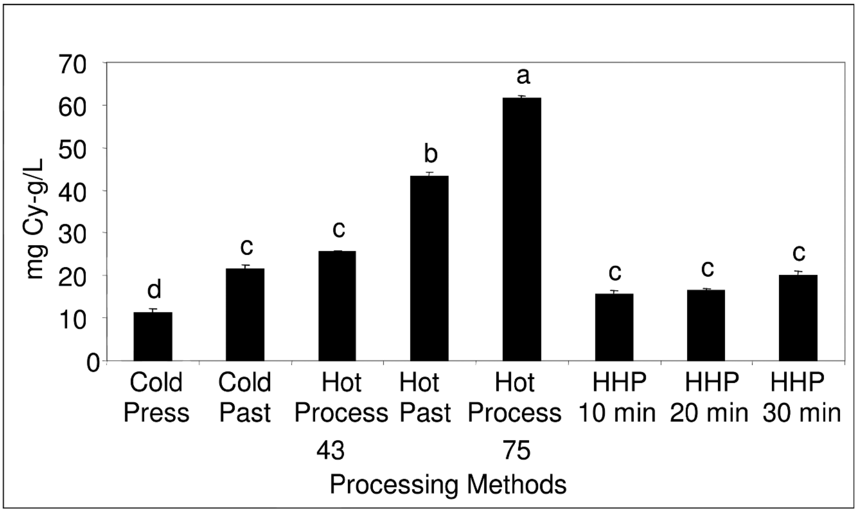


Figure 3. Total anthocyanin content calculated as mg cyanidin-3-glucoside per L of juice in blueberry juices after heat treatments and high pressure processing (HHP). Significant differences between treatments are indicated by different letters ($p<0.05$).

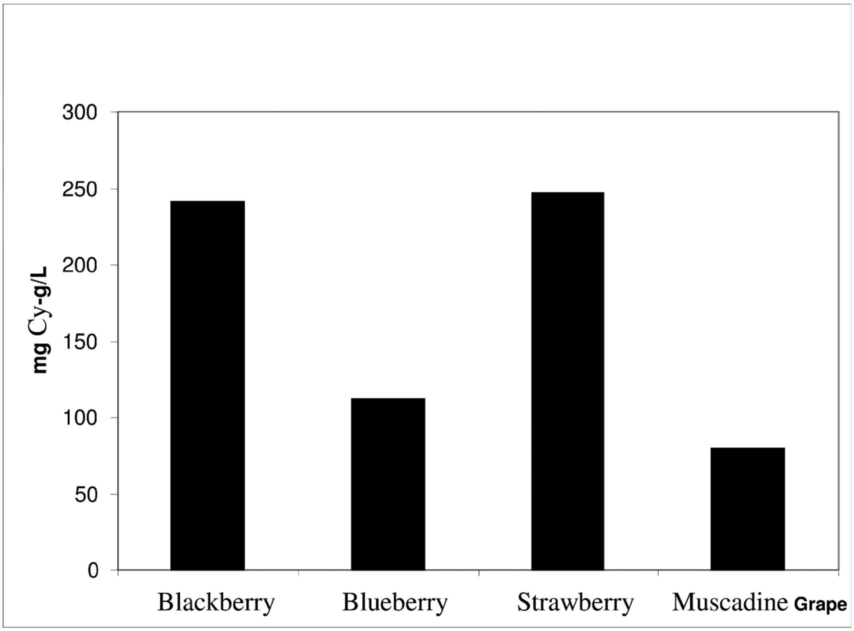


Figure 4. Total anthocyanin content as mg cyanidin-3-glycoside perL of juice from fruit samples after cold pressing.

Downloaded by UNIV OF DELAWARE MORRIS LIB on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch010

Processing Effects on Other Berries and Muscadine Grapes

Although the focus of this research has been on juice from blueberries, other small berries can be processed and treated in similar ways. They can be exploited for their bioactive compounds such as anthocyanin contents (Figure 4). As part of another study, fresh blackberries, strawberries, blueberries and muscadine grapes were cold pressed and the juices were analyzed for their anthocyanin content as previously described for the blueberry juices above. In the case of the blackberries and the strawberries here, they have been proven to be even better sources than blueberries, as would seem logical due to their darker colors. It is to be expected that the extraction of these types of compounds would have effects on the juice flavors and feeling factors such as astringency. It would be interesting to compare the levels of sugars of these juices in comparison with consumer liking studies.

Conclusion

Thermal processing of blueberry juice was found to result in a final product with higher consumer ratings than commercially processed juice when prepared in blends with apple juice. The use of high hydrostatic pressure and enzymatic treatment of blueberries during the juice making process was to improve the release and preserve the color or the bioactive components of the final product. These treatments result in a more efficient process and could be expected to enhance the nutritional and bioactive content as well as the safety of the resulting juice by reducing the microbial load as has been found in other products subjected to this treatment (*13*, *14*). If the juices were found to have a longer shelf life with a greater content of bioactive compounds as a result, this might offset the additional cost. As consumer choices turn toward more products perceived to be "healthy" and "fresh", these processing techniques may prove to be of more importance.

References

1. Mamen, K. Facing Goliath: challenging the impacts of supermarket consolidation on our local economics, communities, and food safety. *The Oakland Inst. Policy Brief* **2007**, *1* (3), 1–8.
2. Nord, M.; Hopwood, H. Higher cost of food in some areas may affect food stamp household's ability to make healthy food choices. *USDA, ERS Econ. Inform. Bull.* **2007**, *29-3*, 1–4.
3. Brunner, E. J.; Mosdøl, A.; Witte, D. R.; Martikainen, P.; Stafford, M.; Shipley, M. J.; Marmot, M. G. Dietary patterns and 15-y risks of major coronary events, diabetes, and mortality. *Am. J. Clin. Nutr.* **2008**, *87*, 1414–21.
4. George, S. M.; Park, Y.; Leitzmann, M. F.; Freedman, N. D.; Dowling, E. C.; Reedy, J.; Schatzkin, A.; Hollenbeck, A.; Subar, A. F. Fruit and vegetable

Downloaded by UNIV OF DELAWARE MORRIS LIB on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch010

intake and risk of cancer; a prospective cohort study. *Am. J. Clin. Nutr.* **2009**, *89*, 347–353.

5. Shin, A.; Lim, S-Y.; Sung, J.; Shin, H-R.; Kim, J. Dietary intake, eating habits, and metabolic syndrome in Korean men. *J. Am. Diet. Assoc.* **2009**, *109*, 633–640.
6. Lorson, B. A.; Melgar-Quinonez, H. R.; Taylor, C. A. Correlates of fruit and vegetable intakes in US children. *J. Am. Diet. Assoc.* **2009**, *109*, 474–478.
7. Center for Disease Control. Fruit & veggies matter: eat a variety of fruits and vegetables everyday. http://www.fruitsandveggiesmatter.gov/ (accessed May 28, 2009).
8. Rodrigo, D.; Van Loey, A.; Hendrickx, M. Combined thermal and high pressure colour degradation of tomato puree and strawberry juice. *J. Food Eng.* **2007**, *79* (2), 553–560.
9. Catsberg, C. M. E.; Kempen-Van Dommelen, G. J. M. *Food Handbook*; Ellis Horwood: New York, 1990; pp 337−338.
10. Flora, L. F. Optimum quality parameters of muscadine grape juices, beverages, and blends. *J. Food Qual.* **1979**, *2*, 219–229.
11. Butz, P.; Tauscher, B. Emerging Technologies: Chemical Aspects. *Food Technol. Res.* **2002**, *35*, 279–284.
12. Oshima, T.; Ushio, T.; Koizumi, C. High pressure processing of fish and fish products. *Trends Food Sci. Technol.* **1993**, *4*, 370–375.
13. Lavinas, F. C.; Miguel, M. A. L.; Lopes, M. L. M.; Valente Mesquita, V. L. Effect of high hydrostatic pressure on Cashew Apple (*Anacardium occidentale* L.) juice preservation. *J. Food Sci.* **2008**, *73*, M273–M277.
14. Buzrul, S.; Alpas, H.; Largeteau, A.; Demazeau, G. Inactivation of *Escherichia coli* and *Listeria innocua* in kiwifruit and pineapple juices. *Int. J. Food Microbiol.* **2008**, *124*, 275–278.
15. Ludikhuyze, L.; Van Loey, A.; Hendrickx, I.; Hendrickx, M. High pressure processing of fruit and vegetables. In *Fruit and Vegetable Processing: Improving Quality*; Jongen, W., Ed.; Woodhead Publishing: Boca Raton, FL, 2002; pp 346−348.
16. Laboissiere, L. H. E. S.; Deliza, R.; Barros-Marcellini, A. M.; Rosenthal, A.; Camargo, L. M. A. Q.; Junqueira, R. G. Effects of high hydrostatic pressure (HHP) on sensory characteristics of yellow passion fruit juice. *Innovative Food Sci. Emerging Technol.* **2007**, *8*, 469–477.
17. Fuleki, T.; Hope, G. W. Effects of various treatments on yield and composition of blueberry juice. *Food Technol.* **1964**, *18*, 568–570.
18. Kalt, W.; McDonald, J. E.; Donner, H. Anthocyanins, phenolics, and antioxidant capacity of processed lowbush blueberry products. *J. Food Sci.* **2000**, *65*, 390–393.
19. Buchert, J.; Koponen, J. M.; Suutarinen, M.; Mustranta, A.; Lille, M.; Törrönen, T.; Poutanen, K. Effect of enzyme-aided pressing on anthocyanin yield and profiles in bilberry and blackcurrant juices. *J. Sci. Food Agric.* **2005**, *85*, 2548–2556.
20. Diano, N.; Grimaldi, T.; Bianco, M.; Rossi, S.; Gabrovska, K.; Yordoanova, G.; Godjevargova, T.; Grano, V.; Nicolucci, C.; Mita, L.; Benevenga, U.; Conciglia, P.; Mita, D. G. Apple juice clarification by

immobilized pectolytic enzymes in packed or fluidized bed reactors. *J. Agric. Food Chem.* **2008**, *56*, 11471–11477.

21. Santin, M. M.; Treichel, H.; Valduga, E.; Cabral, L. M. C.; DiLuccio, M. Evaluation of enzymatic treatment of peach juice using response surface methodology. *J. Sci. Food Agric.* **2008**, *88*, 507–512.
22. Valcárcel, M. C.; Palacios, V. Influence of 'Novarom G' pectinase β-glycosidase enzyme on the wine aroma of four white varieties. *Food Sci. Technol. Int.* **2008**, *14*, 95–102.
23. Koponen, J. M.; Buchert, J.; Poutanen, K. S.; Törrönen, A. R. Effect of pectinolytic juice production on the extractability and fate of bilberry and black currant anthocyanins. *Eur. Food Res. Technol.* **2008**, *227*, 485–494.
24. U.S. Department of Agriculture, National Agricultural Statistic Service. *Non Citrus Fruits and Nuts. 2008 Survey*. FrNt 1-3, 2009.
25. Helrich, K., Ed. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed.; Association of Official Analytical Chemists: Arlington, VA, 1990.
26. Giusti, M. M.; Wrolstad, R. E. Characterization and measurement of anthocyanins by uv-visible spectroscopy. In *Current Protocols in Food Analytical Chemistry*; Wrolstad, R. E., Ed.; John Wiley and Sons: New York, 2001; Unit F1.2.1-F1.2.13.
27. Amerine, M. A.; Ough, C. S. *Methods for Analysis of Musts and Wines*; John Wiley and Sons: New York, 1980; pp 181−184.
28. Prior, R. L.; Hoang, H.; Gu, L.; Wu, X.; Bacchiocca, M.; Howard, L.; Woodill, M.; Huang, D.; Ou, B.; Jacob, R. Assays for hydrophilic and lipophilic antioxidant capacity (Oxygen Radical Absorbance Capacity ($ORAC_{FL}$) of plasma and other biological and food samples. *J. Agric. Food Chem.* **2003**, *51*, 3273–3279.
29. Huang, D.; Ou, B.; Prior, R. L. The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.* **2005**, *53*, 1841–1856.
30. U.S. Department of Agriculture, Nutrient Date Laboratory. Oxygen radical absorbance capacity (ORAC) of selected foods-2007. http://www.ars.usda.gov/SP2UserFiles/Place/12354500/Data/ORAC/ORAC07.pdf (accessed May 29, 2009).
31. Robicaud, J. L.; Noble, A. C. Astringency and bitterness in selected phenolics in wine. *J. Sci. Food Agric.* **1990**, *53*, 343–353.
32. Lea, A. G. H.; Arnold, G. M. The phenolics of ciders: bitterness and astringency. *J. Sci. Food Agric.* **1978**, *29*, 478–483.
33. Strauss, C. R.; Wilson, B.; Anderson, R.; Williams, P. J. Development of precursors of C_{13}-norisoprenoid flavorants in Riesling grapes. *Amer. J. Enol. Vitic.* **1987**, *38*, 23–27.
34. Suthanthangjai, W.; Kajda, P.; Zabetakis, I. The effect of high hydrostatic pressure on the anthocyanins of raspberry (*Rubus idaeus*). *Food Chem.* **2005**, *90*, 193–197.

Downloaded by UNIV OF DELAWARE MORRIS LIB on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch010

Downloaded by STANFORD UNIV GREEN LIBR on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch011

Chapter 11

Enhanced Stability of Citral in Juice Beverages by Applying Cyclodextrin Micro Emulsion Technology

Ken Strassburger,* William Startup, Victor Levey, Tracey Mattingly, Judy Briggs, Joy Harrison, and Terry Wilson

Cargill Flavors Systems, 10311 Chester Road, Cincinnati, Ohio 45215, USA
***Ken_Strassburger@cargill.com**

Lemon oils and flavors are very unstable in the acidic media, typical in most juice and juice based beverages. Not only is there a dramatic change in profile with the loss of fresh lemon character, the development of off notes is more troublesome and problematic. Other significant changes occur due to photo stability (sun-struck) and packaging interaction, which further degrades the final consumer quality. The application of *β*-cyclodextrin flavor complexes in designing beverage systems can eliminate off note development, maintain true lemon profile, minimize packaging interaction in glass or PET and offer a high degree of photo-stability without employing additional anti-oxidants or expensive package engineering.

Introduction

Citral stability in aqueous acidic beverage is an age old problem that the food, flavor and beverage industry has spent hundreds or thousands of man years trying to solve (*1*–*3*). Not only is this stability issue responsible for the loss of fresh lemon character; powerful off-notes are generated detracting from product acceptance. The accepted mechanism of off-note formation as summarized by Ueno (*1*) is detailed in Figure 1. Additionally, Schieberle and Grosch (*2*) propose a mechanism whereby in acidic media, geranial (E configuration) rapidly isomerizes to neral (Z configuration) which, in turn, cyclizes to form the reactive intermediates

In Flavors in Noncarbonated Beverages; Da Costa, N., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

Downloaded by STANFORD UNIV GREEN LIBR on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch011

p-menthadien-8-ols. Freeberg (*3*) completes the picture by studying beverage systems with and without citral.

Our data confirms much of the above references with lemon beverage stability; however, the identity and concentration of these components varies dramatically depending on source material (pure citral or lemon oils), beverage preparation (organic in water or emulsions), sample preparation and analytical conditions. For example, compared to Figure 1, we easily find p-cymene, but no α-terpineol in citral studies, yet both are present in lemon oil studies as discussed by Freeburg (*3*). In our studies, we can easily confirm p-cresol by sensory testing, yet it needs to exceed 2ppm to be detectable by SPME, which is 100X its taste threshold. Many do not agree that α-terpineol is a defect, others site it is slightly objectionable. Consumers are accustomed to a lemon-lime profile. Most however, do agree that p-cresol, p,α-dimethylstyrene and p-methylacetophenone are the major off character defects to be addressed. Odor thresholds in water of these well known compounds are listed in Table I. We will also demonstrate that a similar pathway is responsible for off-note formation during UV exposure (sun-struck phenomenon). The rate of citral degradation has also been reported as accelerated in gas permeable packaging such as polyethylene (*5*).

The typical free radical scavenger antioxidants, such as BHA, BHT, tocopherol, etc have not proved effective in aqueous systems. Ueno (*4*) and Banks (*5*) report some success inhibiting the formation of p-methyl-acetophenone using water soluble extracts such as theaflavins and rosemarinic acid, respectively; however, these only seem to block the oxidation of p,α-dimethylstyrene to p-methylacetophenone. A more generalized protection scheme that protects true lemon profile and maintains flavor intensity is still highly sought after.

Several years earlier we had begun a program to study cyclodextrin encapsulated flavors and oils for extended shelf life. During the course of preparing these materials we noticed the novel and significant activity of the cyclodextrins in solution and saw this property as a means to stabilize citral directly (*6*).

Cyclodextrins

The cyclodextrins (CD) have been know for many years and are very adequately described in the literature, but basically, cyclodextrins are natural molecules produced by the action of enzymes on corn starch (Figure 2) and consist of 6, 7, or 8 glucose molecules arranged in a 3-D torrid which have a hydrophilic exterior with a hydrophobic interior. They can function as hydrophobic wells or reservoirs for certain organic molecules in water and are generally used as encapsulating agents of flavor, fragrance or drug molecules (guests) The guest molecule is held in the center cyclodextrin cavity by van der Waals forces and hydrophobic – hydrophilic interaction. Typically, a guest-CD complex forms in solution and is dried to lock the guest-CD complex as a dry powder. The actual formation is governed by thermodynamic and solubility parameters with water being a necessary ingredient to facilitate transport of the guest molecule into the

Downloaded by STANFORD UNIV GREEN LIBR on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch011

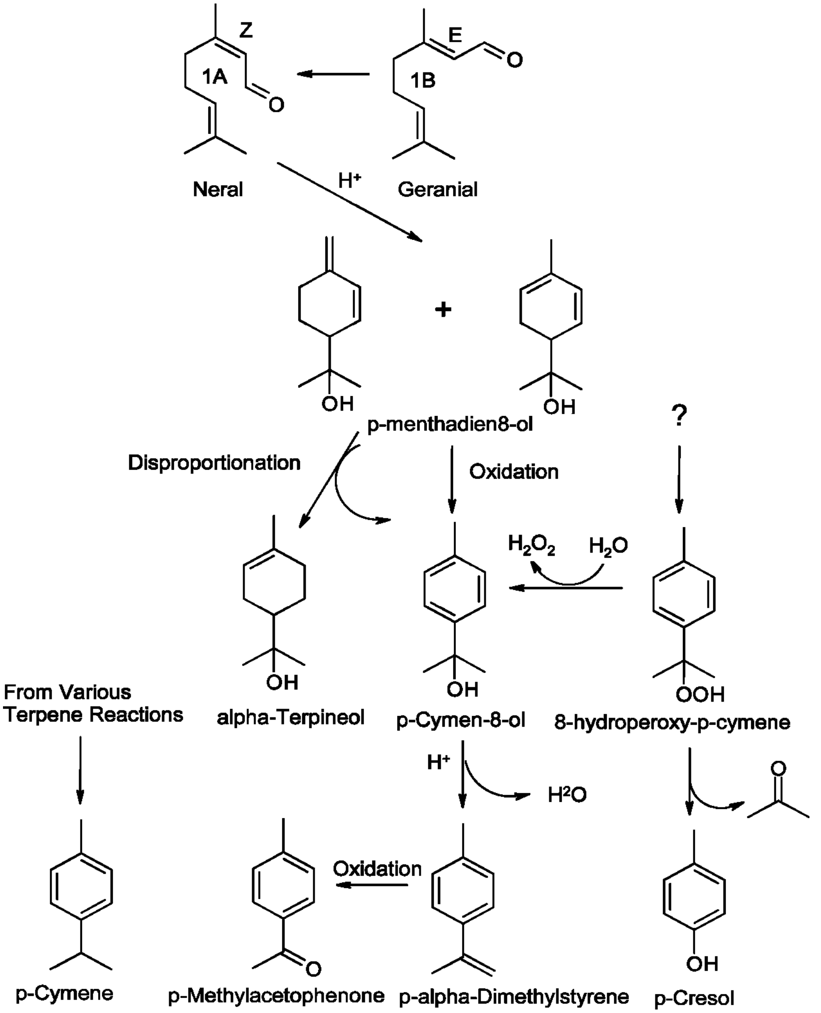

Z
1A
Neral
E
1B
Geranial
H+
p-menthadien8-ol
OH
OH
?
Disproportionation
Oxidation
H_2O_2
H_2O
From Various Terpene Reactions
alpha-Terpineol
p-Cymen-8-ol
8-hydroperoxy-p-cymene
OOH
H+
H2O
Oxidation
p-Cymene
p-Methylacetophenone
p-alpha-Dimethylstyrene
p-Cresol
O

Figure 1. The reaction pathway from citral to potent off notes. (Modified from Ueno reference (2)).

cyclodextrin cavity, however, the activity of cyclodextrins in solution has not been previously discussed or exploited commercially.

In the case of citral, specifically geranial, there can also be interaction of the "E" configured aldehyde with the exterior hydroxyl groups of the cyclodextrin which results in the loss of an additional degree of freedom as well as the hydrophobic interaction that thermodynamically favors binding or resonance stabilization of citral in solution. This concept is further developed below in a publication from Lantz (*7*) in which he coins the term pseudophase to describe the behavior of organic solvents in cyclodextrin solutions. K_H is Henery's constant expressing the relationship between a solute of concentration C_S and its vapor pressure P_S , with Kp_2 representing the equilibrium established between the free

Downloaded by STANFORD UNIV GREEN LIBR on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch011

Table I. Organoleptic Properties (odor thresholds in water from ref (*4*))

Off-Odorant	*Threshold (µg/L)*	*Taste Character*
p-Cymene	6.2-150	Gasoline like
p-Cresol	2.3-55	Phenolic-burnt
p-Methylacetophenone	19-24	Bitter almond
p,α-Dimethylstyrene	85	Green-plastic like
p-Cymen-8-ol	22500	Sharp green–berry/cherry

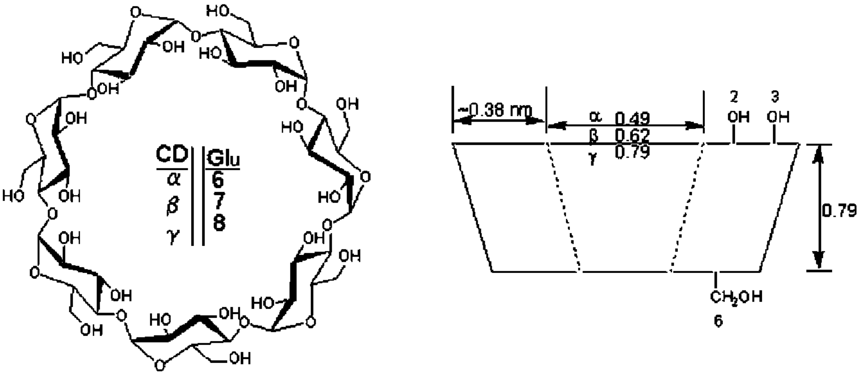


Figure 2. Cyclodextrin models showing geometry and physical dimensions of the hydrophobic core and hydrophilic exterior.

solute and the solute-cyclodextrin complex and K_{p1} showing the existence of direct solute-cyclodextrin complex and vapor phase equilibrium. We have utilized and extended this model to more completely describe the interaction of citral or citral containing flavors in beverage systems.

It is necessary to distinguish between static and dynamic systems as applied to beverage applications. Using the nomenclature from Figure 3, in a bottled beverage (static system), K_{P2} predominates. Upon opening and consumption, a more dynamic system is established with K_H and K_{P1} predominating. Experimentally it can be shown that K_{P2} is heavily influenced by both log(P), the octanol-water partition coefficient, and binding efficiency, such that citral is preferentially protected even with a large excess of limonene. Thus, we can exploit the aqueous equilibrium established by the addition of cyclodextrin as the flavor carrier to protect flavor quality. Moeder (*12*) has reported on the direct measurement of the thermodynamic properties of cyclodextrins and terpenes in solution via their use as mobile phase modifiers in HPLC.

As is the case with surfactants, there is a critical micelle concentration (CMC) for these effects to predominate the beverage system. In the case of β-cyclodextrin, this concentration is between 0.05% and 0.2%. On tasting, the necessary CMC falls below the equilibria value and flavor molecules cannot re-establish the resonance stabilized pseudophase and are, therefore, available for taste.

Downloaded by STANFORD UNIV GREEN LIBR on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch011

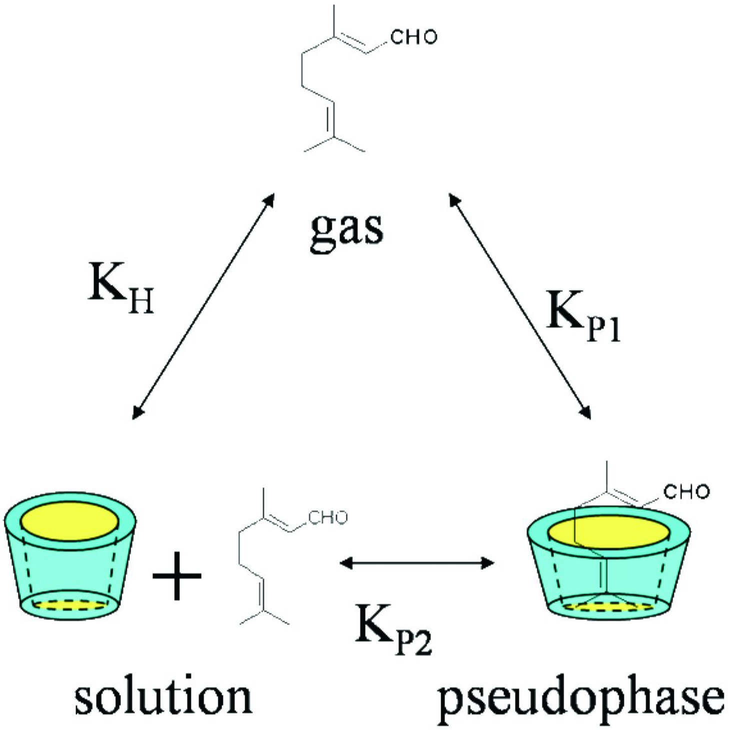


Figure 3. Cyclodextrin – guest equilibrium in solution (geranial "E" shown).

By viewing the beverage as a static system at equilibrium or steady state, certain other advantages become apparent. Where n_S^{total} represents the number of moles of flavor molecule partitioned between n_S^G, n_S^W and n_S^{CD} ; gas phase, water phase, and pseudophase respectively:

- $n_S^{total} = n_S^G + n_S^W$ **without cyclodextrin and,** (1)

- $n_S^{total} = n_S^G + n_S^W + n_S^{CD}$ **with cyclodextrin** (2)

for a beverage at steady state, the mass balance becomes:

- $n_S^{total} = n_S^G + n_S^W + n_S^{CD} - f_{(P)}$ (3)

where $f_{(P)}$ represents migration through a barrier and can be measured. For a beverage with a significant log(P) flavor, such as citrus:

- $n_S^{CD} >> n_S^W > n_S^G > f_{(P)}$ (4)

Downloaded by STANFORD UNIV GREEN LIBR on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch011

Table II. Beverage System 1 Citral Only (pH=2.6)

Ingredient	*Control Sample "A"*	*β-Cyclodextrin "B"*	*β-Cyclodextrin "C"*
DI Water	1000	1000	1000
Sugar	100	100	100
Citric acid	5	5	5
Citral 0.1% in Ethanol	1		
Citral 0.1% in BCD		1	
Citral 0.05% in BCD			2

Table III. Beverage System 2 Lemon Emulsion System (pH=2.6)

Ingredient	*Control Sample*	*β-Cyclodextrin 1*
DI Water	1000	1000
Sugar	100	100
Citric acid	5	5
Citral 0.1% in Ethanol	1.8	
Citral 0.1% in BCD		1.8
Citral free Lemon Emulsion	1.0	1.0

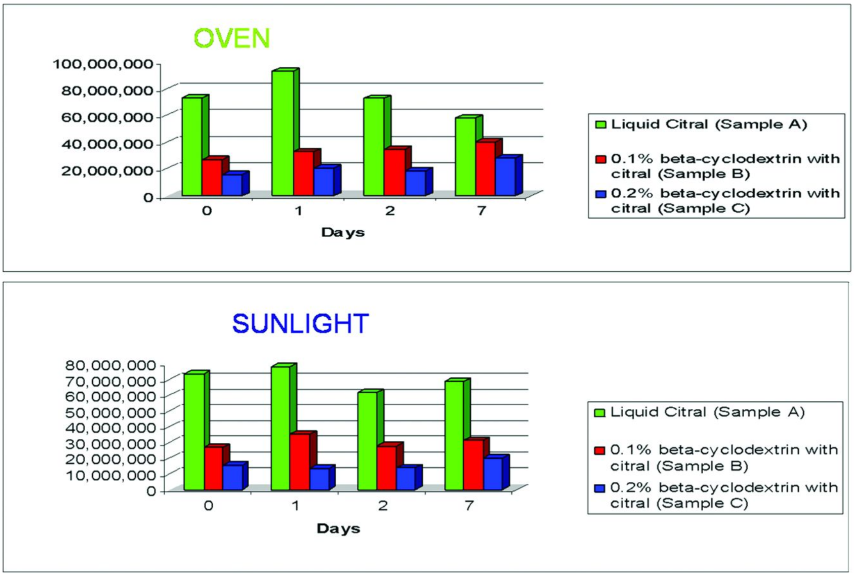


Figure 4. SPME analysis of p-menthadien-ol(s) – concentrations are reported in raw area counts.

Downloaded by STANFORD UNIV GREEN LIBR on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch011

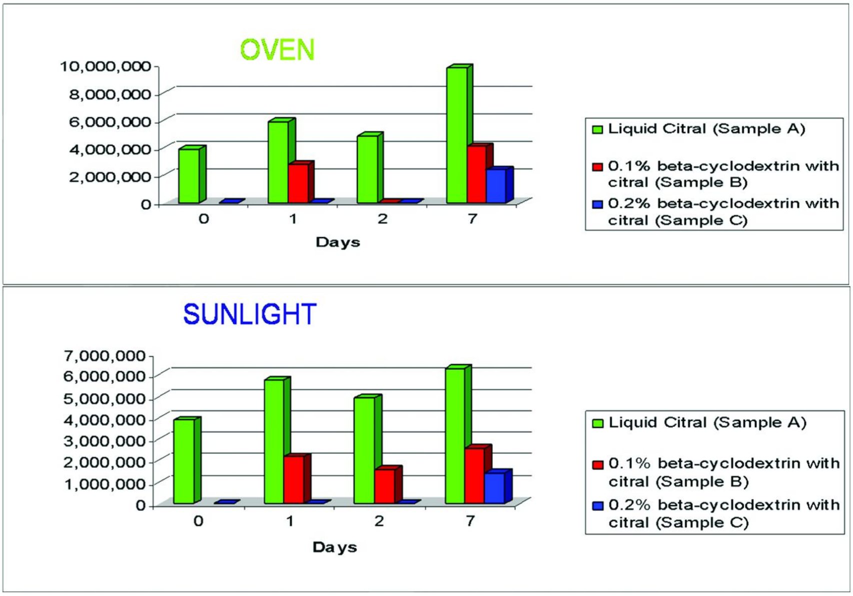


Figure 5. SPME results for p-cymen-8-ol concentration over time.

Pseudophase development should have a dramatic impact on volatile retention or flavor intensity of the beverage product. In the case of citrus products where $\log(P)_{citral} > 3.0$, the flavor materials that had been most difficult to stabilize due to "ringing-out" or adsorption into packaging in the past are the most impacted (favorably) in the cyclodextrin systems. The analysis of absorbed flavor in container material, especially PET, low density polyethylene and lid seals is easily accomplished with headspace techniques such as SPME.

Specifically addressing the acid stability of citral, we can take a kinetic view of the importance of a pseudophase development from an aqueous equilbria experiment. Historically, citral was often removed from the lemon essential oils prior to use, which dramatically alters the flavor profile. The fragrance industry substitutes a nitrile group replacing the reactive aldehyde; however, this is not allowed in foods and flavors. Adjusting the pH is also not a viable option without a dramatic change in taste profile:

$$\frac{[offnote]^{z}}{[citral]^{x} \times [H^{+}]^{y}} = \kappa \qquad (5)$$

$$[offnote] = \begin{array}{l} \kappa[p-mentha-dien-8-ol] \rightarrow \kappa[p-cymen-8-ol] \rightarrow \\ \kappa[p-\alpha-dimethylstyrene] \rightarrow \kappa[p-methylacetophenone] \end{array} \qquad (6)$$

$$\left[offnote\right] = \begin{array}{l} \kappa\left[p - mentha - dien - 8 - ol\right] \rightarrow \kappa\left[p - cymen - 8 - ol\right] \rightarrow \\ \kappa\left[8 - hydroxyperoxy - p - cymene\right] \rightarrow \kappa\left[p - cresol\right] \end{array} \tag{7}$$

We propose in expression (5), that the total concentration of off-note development can be controlled by modifying the molar concentration of free citral in solution through pseudophase formation. It also follows that each reaction path (eq 6) and (eq 7) to the potent compounds p,α-dimethylstyrene, p-methylacetophenone and p-cresol should also be significantly reduced, again by controlling and limiting reactant concentrations. The formation constants vary with temperature and have been discussed by Freeburg (*3*). Cravotto has recently reviewed cyclodextrins as food additives (*8*). Lopez-Nicholas has reported of the prevention of browning in juice using natural and modified cyclodextrins (*9–11*).

Materials and Methods

Two types of lemon beverage applications were studied utilizing cyclodextrin technology. The first and harshest contains 10ppm citral (only) in a sugar, acid, water system. At this level and beverage construction, citral is completely soluble and most reactive. A second, emulsion system, is presented for comparison; the composition of both systems is given in Tables II and III. Citral and off-note standards were obtained from Sigma-Aldrich Chemicals; cyclodextrins were purchased from Wacker Chemicals, lemon flavors and citral-*β*-cyclodextrin (BCD) complexes were prepared by Cargill Flavor Systems. The citral-*β*-cyclodextrin complex was prepared according to reference (*6*) at 10wt% and blended with excess *β*-cyclodextrin to achieve either 0.1wt% or 0.05wt% citral which was added to the beverage providing the proper flavor level for citral and a predetermined amount of *β*-cyclodextrin necessary for stabilization. Ethanol (0.1%) was added to the *β*-cyclodextrin containing beverages to assure equal solubility and headspace partitioning / sampling for each sample.

The analytical and storage conditions for Beverage System 1, packaged in GLASS are:

- Beverages were pasteurized on a Micro Thermics UHT pilot unit
- Analysis by SPME using a 2cm carboxen fiber (Supleco) with 1ml sample volume and 20 min exposure @ 50°C
- GC/MS analysis on Pegasus III TOF using a 60m –x- 0.25mm DB-5MS (0.5μm film thickness) with He carrier @ 1.5ml/min, desorbed sample was cryo-focused @ -70°C
- Analysis in triplicate using latin square protocol:
    - A B C
    - B C A
    - C A B
- Peak intensity measurements are reported in raw area counts
- Oven samples were stored @ 32°C
- Light samples were stored in our lab windows and rotated daily:

Downloaded by STANFORD UNIV GREEN LIBR on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch011

Downloaded by STANFORD UNIV GREEN LIBR on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch011

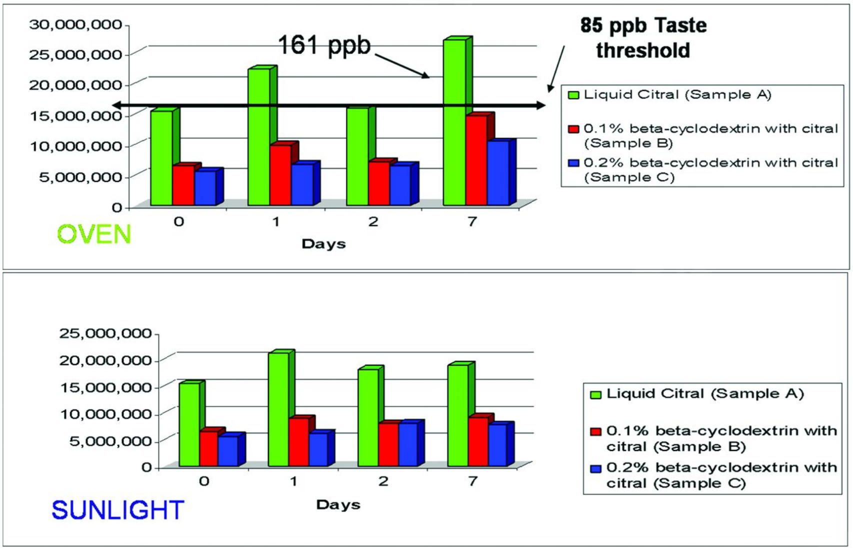


Figure 6. SPME results for p,α-dimethylstyrene formation, the indicated threshold is obtained from an analyzed taste standard.

- Max light = 1,728 lum/ft$_2$
- Min light = 0
- Ave light over 7 days = 271.5 lum/ft$_2$
- Ave temperature = 24°C

Results

The SPME analysis of the stored beverages are examined in Figures 4−7, starting with development of the key intermediate, the p-mentha-dien-ol(s), two isomers, that form very quickly during pasteurization especially in an un-protected system (Figure 4). Analysis of samples (data not shown) prior to pasteurization shows only citral. The threshold values stated are from reference (*4*), with both the analytical standards and sensory standards prepared fresh as needed. The standards are analyzed under identical conditions to generate quantitation levels and to plot actual threshold values with the beverage analytical presentation. Because of the difficulty obtaining high purity standards only p,α-dimethylstyrene (Figure 6) and p-methylacetophenone (Figure 7) were calibrated.

There is very little information in the literature that describes the photo stability of citral, however our data demonstrates that it appears to generally follow the traditional acid catalyzed pathway; the sun-struck phenomenon being a combination of heat with UV exposure. No new or extraneous compounds were identified during the course of our studies.

Downloaded by STANFORD UNIV GREEN LIBR on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch011

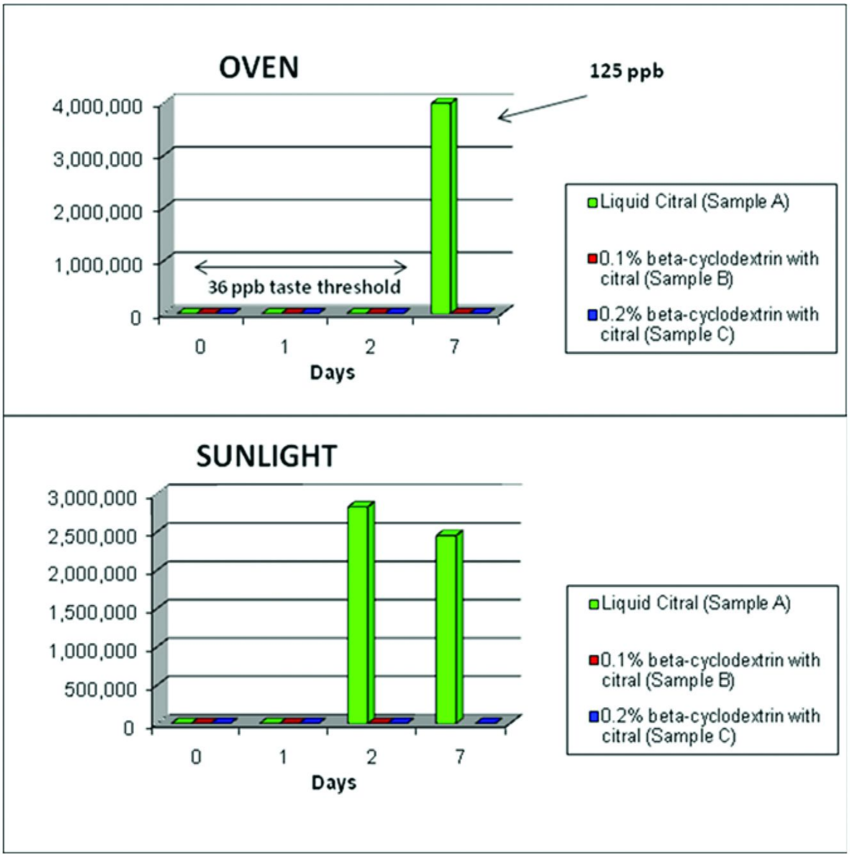


Figure 7. SPME results for p-methylacetophenone formation, as before with the p,α-dimethylstyrene data; the threshold indicated is an analyzed sample.

In both the oven and sunlight sample, the β-cyclodextrin protected samples show reduced off-note formation throughout the pathway. The concentration of p,α-dimethylstyrene is held below its taste threshold with no p-methyl-acetophenone observed in the protected samples.

A good theory must be proven with sensory as well as analytical data. Expert sensory (descriptive) panels were conducted at the first and seventh day on each same sample set. As mentioned, off-note as well as positive attribute organoleptic standards were prepared in sugar, citric acid solution at known concentrations, approximately at published threshold levels and ten times threshold. A six member trained panel was utilized. The results are summarized in Figure 8 and Figure 9.

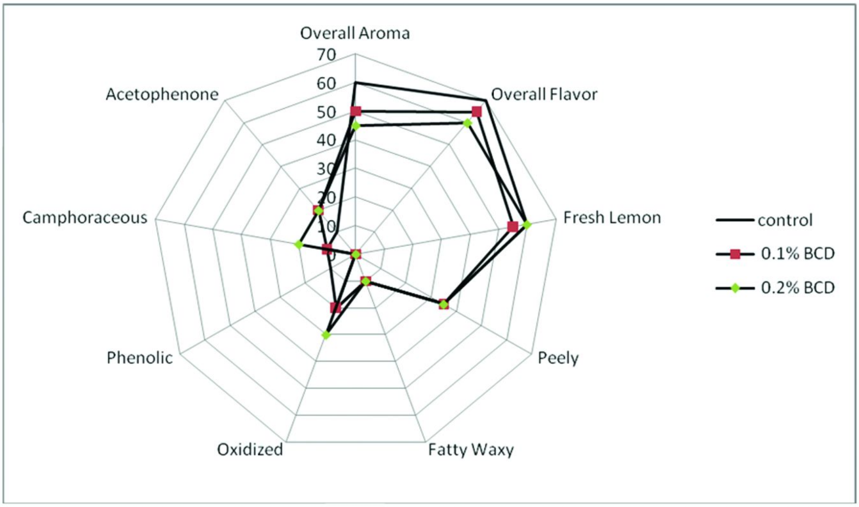


Figure 8. Sensory evaluation of protected and unprotected beverages at day 1.

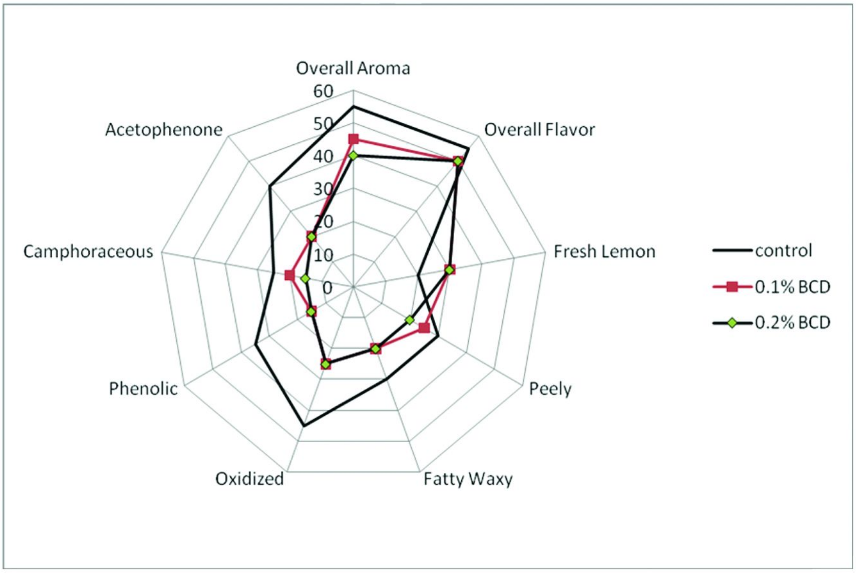


Figure 9. Sensory evaluation of protected and unprotected beverages at day 7.

At day 7 the control (unprotected) beverage was considered oxidized. The overriding sensory attributes being oxidized, phenolic and almond-acetophenone like character. Both of the β-cyclodextrin protected beverage samples were considered still acceptable, but with slightly reduced fresh lemon or citral note. Analytical data demonstrates that significantly more citral is still present in the protected system than in the unprotected versions as our pseudophase theory

Downloaded by STANFORD UNIV GREEN LIBR on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch011

Downloaded by STANFORD UNIV GREEN LIBR on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch011

would suggest. It does however take some finite time to release or re-equilibrate to free citral. We demonstrate this phenomenon Figure 10 below where we have taken the 7 day stability samples, analyzed as previously described, and as a 50% dilution in water. One would expect to see half the concentration of citral in the dilution; which is the case in the unprotected system. The *β*-cyclodextrin protected system however, maintains almost the same level of citral on dilution. Extending this phenomenon to a theory of taste, on a very simplistic level, *β*-cyclodextrin provides an extended release of flavor while maintaining significant protection of the flavor profile. The difference in p-cymene concentration shown below is one example. Additional studies are necessary to profile concentration effects and flavor character in these systems and especially initial release and flavor onset time in the mouth.

Emulsion Systems

A more complete and typical flavored beverage was constructed as detailed in Table III utilizing a citral-free emulsion weighted with ester gum. As mentioned earlier, beverage preparation can play a major role in stability. In this example we compare beverage prepared from lemon emulsions, again in sugar, acid and water. The emulsion is a citral free lemon flavor, as described by Freeburg, freshly made to prevent storage issues; citral was added as an ethanol cut (control) or as a *β*-cyclodextrin complex; the citral content of both beverages is 15ppm. After pasteurization, each beverage is packaged in 12oz PET bottle and stored at 32°C for three weeks (21 days) before tasting and analysis.

At three weeks, the sensory profile of the lemon emulsion beverages was characterized as oxidized, phenolic and acetophenone-like for the control while the *β*-cyclodextrin protected beverage maintains the fresh lemon character with a significant lack of off-notes (Figure 11). In this situation, the *β*-cyclodextrin can be view as a secondary antioxidant that scavenges compounds that leach from the suspended emulsion droplets. It is highly probable that the emulsion formation itself adds some stabilization due to formation of hydrophobic shielding in the droplets as long the suspension is homogeneous. However, as the emulsion begins to break, acid sensitive compounds are increasingly exposed to the oxidative environment of the beverage. As seen in the previous examples, *β*-cyclodextrin can be a highly active stabilizing factor.

The systems under study become very complex and numerous factors need to be explored. In the prior examples we took a purely organic chemistry approach to off note development, several added applications follow.

We mentioned, briefly before, that α-terpineol represents a profile change in fresh lemon type beverages. Not all would agree that this change is for the worse and consumers are accustomed to lemon-lime instead of a true lemon profile in beverages. However, the formation of *α*-terpineol does arise from acid mediated reactions of various lemon components as shown in Figure 1 and can be dramatically impacted with the use of *β*-cyclodextrin. The data in Figure 12 is taken from the emulsion study under discussion and is reported as the relative percentage of total flavor volatiles. It becomes apparent that the impact on the

Downloaded by STANFORD UNIV GREEN LIBR on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch011

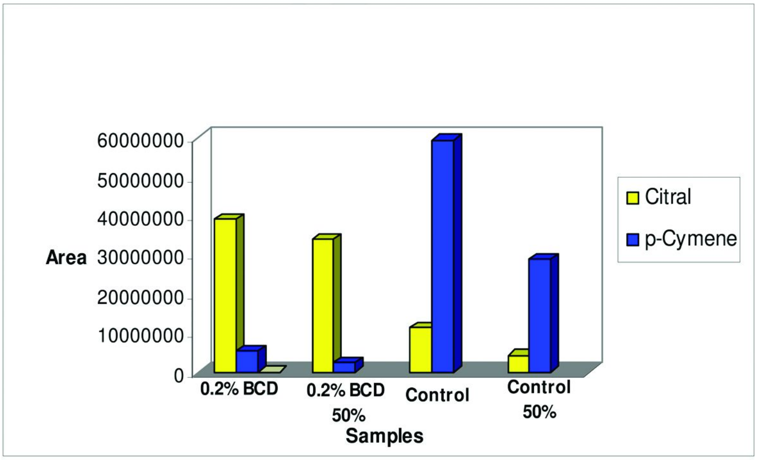


Figure 10. A demonstration of protective and pseudophase (storage) effect of β-cyclodextrin with citral by diluting the sample 50%, p-cymene is uneffected.

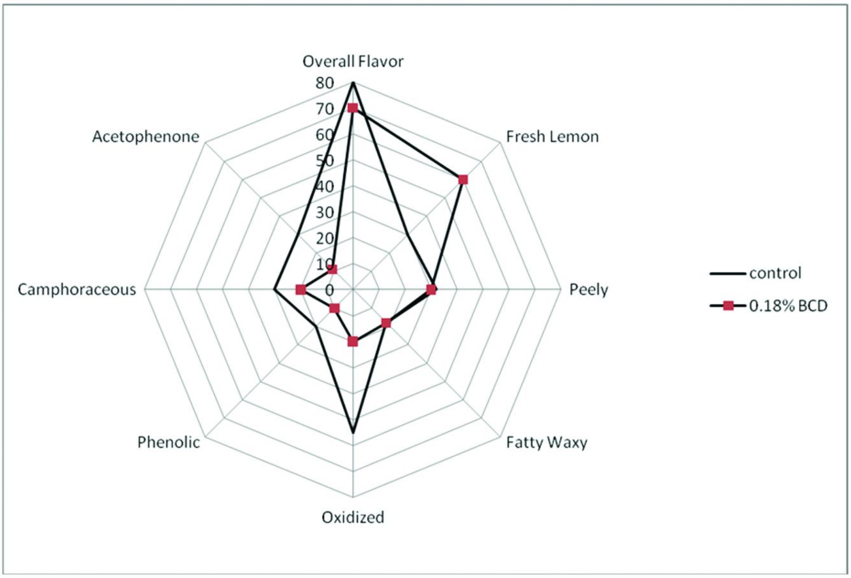


Figure 11. Sensory evaluation of a lemon emulsion system with β-cyclodextrin after 3 weeks @ 32°C.

beverage profile and quality can become significant over time. While many consumers are accustomed to a lemon-lime profile, the use of β-cyclodextrin allows the creation of a truly fresh lemon only if desired. This data also strongly supports the case that multiple reactions occur in storage and more general protection schemes are needed. Of course, the use of β-cyclodextrin with lime (α-terpineol) would maintain a fresh lime without off notes and more importantly,

Downloaded by STANFORD UNIV GREEN LIBR on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch011

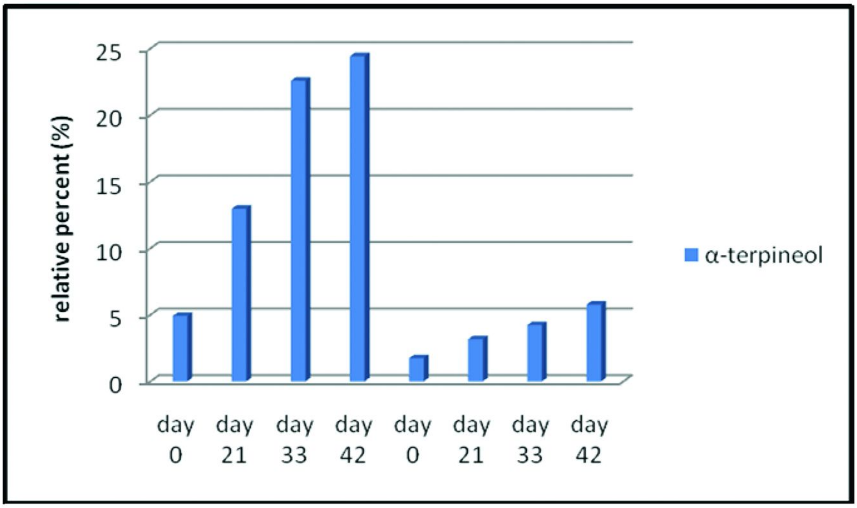


Figure 12. α-Terpineol formation in a lemon emulsion plus citral formulation, control sample is plotted on the left with the β-cyclodextrin sample on the right.

β-cyclodextrin: citral, *α*-terpineol, vanillin or benzaldehyde complexes could be used to stabilize cola products.

An added benefit that has not been previously documented is the phenomenon of overcoming package interaction, both migration and absorption, with *β*-cyclodextrin. The same complex formation and equilibrium that prevents off note formation also confers a resonance stabilized thermodynamic effect that overcomes migration and permeability when a beverage is in contact with a polymer film or container. As shown in Figure 13, the *β*-cyclodextrin stabilized beverage maintains much higher intensity over the course of the 42 day analytical study. The growth of the previously defined off notes that increase over time, and more importantly, grow relative to the available flavor intensity of the product significantly modifying the overall taste profile. The off note concentrations above are multiplied by a scalar of 10 for clarity and should not be considered as relative percentages; however, a clear trend is visible. Package design and engineering is a costly piece of delivering the flavor profile to the shelf; the use of cyclodextrins can have a significant impact on product and package performance.

Conclusions

We have demonstrated that cyclodextrins and flavor cyclodextrin complexes can be very active in beverage systems and can provide novel stabilization to important classes of flavor compounds and systems. The compounds that have previously been the most difficult to stabilize are readily accommodated by the use of cyclodextrin and could be extended to other product types. While the subject of this report focused on citral, it is highly likely that a number of terpene reactions are involved in the deterioration of lemon and citrus beverages that can

Downloaded by STANFORD UNIV GREEN LIBR on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch011

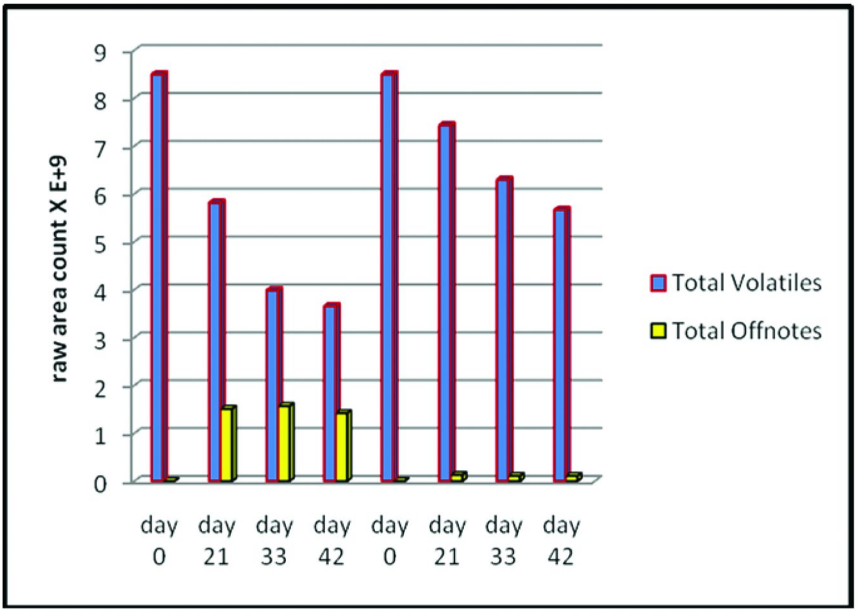


Figure 13. Total flavor volatiles (off-notes and positive attributes) by SPME from the emulsion example Control sample is plotted on the left with the β-cyclodextrin sample on the right.

be improved with the application of β-cyclodextrin. This protection is the direct result of a pseudophase development or resonance stabilization of the organic flavor molecules in solution. In practice, the amount of cyclodextrin employed can suppress the initial flavor intensity so a creative effort is still required. The cyclodextrins also have certain regulatory constraints that must be considered.

It is also very interesting, from a physical chemistry prospective that this behavior is exclusive to *β*-cyclodextrin, which has the poorest aqueous solubility. Attempts using *α*-cyclodextrin, with six glucose molecules constructing the center torid and much greater water solubility, or hydroxypropyl-*β*-cyclodextrin, which has the same seven glucose torid and enhanced solubility over *β*-cyclodextrin; have demonstrated no stabilizing effects in aqueous media.

Acknowledgments

This project was conducted over several years and supported by numerous individuals. I would like to thank Bill Startup, Manager of Analytical Chemistry; Victor Levey and Tracy Mattingly for flavor and beverage development; Judy Briggs, Sensory Manager; Joy Harrison for analyzing numerous samples; and Terry Wilson for organizing the sample protocols.

Downloaded by STANFORD UNIV GREEN LIBR on June 22, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch011

References

1. Ueno, T.; Masuda, H.; Ho, C. T. Formation Mechanism of p-Methylacetophenone from Citra via a tert-Alkoxy Radical Intermediate. *J. Agric. Food Chem.* **2004**, *52*, 5677–5684.
2. Schieberle, P.; Grosch, W. Identification of Potent Flavor Compounds in Aqueous Lemon Oil/Citric acid Emulsions. *J. Agric. Food Chem.* **1988**, *36*, 797–800.
3. Freeburg, E. J.; Mistry, B. S.; Reineccius, G. A.; Scire, R. Stability of Citral-Containing and Citralles Lemon Oils in Flavor Emulsions and Beverages. *Perfum. Flavor.* **1994**, *19*, 23–32.
4. Ueno, T.; Kiyohara, S.; Ho, C. T.; Masuda, H. Potent Inhibitory Effects of Black Tea Theaflavins on Off-Odor Formation from Citral. *J. Agric. Food Chem.* **2006**, *54*, 3055–3061.
5. Banks, V. U.S. Patent 6,638,555, October 28, 2003.
6. Strassburger, K. WO Patent Application 137958, 2006.
7. Lantz Use of The Three Phase Model and Headspace Analysis for the Facile Determination of All Partition/Association Constants for Highly Volatile Solute-Cyclodextrin-Water Systems. *Anal. Bioanal. Chem.* **2005**, *383*, 160–166.
8. Cravotto Cyclodextrins as Food Additives and in Food Processing. *Curr. Nutr. Food Sci.* **2006**, *2*, 343–350.
9. Lopez-Nicolas, J.; Nunez-Delicado, E.; Sanchez-Ferrer, A.; Garcia-Carmona, F. Kinetic Model of Apple Juice Browning in the Presence of Cyclodextrins. *Food Chem.* **2007**, *101* (3), 1164–1171.
10. Lopez-Nicolas, J.; Garcia-Carmona, F. Use of Cyclodextrins as Secondary Antioxidants to Improve the Color of Fresh Pear Juice. *J. Agric. Food Chem.* **2007**, *55* (15), 6330–6388.
11. Lopez-Nicolas, J.; Perez-Lopez, A.; Carbonell-Barrachina, A.; Garcia-Carmona, F. Use of Natural and Modified Cyclodextrins as Inhibiting Agents of Peach Juice Enzymatic Browning. *J. Agric. Food Chem.* **2007**, *55* (13), 5312–5319.
12. Moeder, C.; O'Brien, T.; Thompson, R.; Bicker, G. Determination of stoichiometric coefficients and apparent formation constants for α- and β-CD complexes of terpenes using reversed-phase liquid chromatography. *J. Chromatogr., A.* **1996**, *736*, 1–9.

Downloaded by PENNSYLVANIA STATE UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch012

Chapter 12

Odorant Release from Alcoholic Beverages

Andrew J. Taylor,*,[1] Maroussa Tsachaki,[1] Ricardo Lopez,[2] Cecile Morris,[1] Vicente Ferreira,[2] and Bettina Wolf[1]

[1]Division of Food Sciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK
[2]Universidad Zaragoza, Faculty of Science, Department of Analytical Chemistry, E-50009 Zaragoza, Spain
***andy.taylor@nott.ac.uk**

Odorant release from alcoholic beverages creates the pleasant odor before drinking as well as contributing to the overall flavor during consumption, thus an important feature for the consumer. Odorant release between a gas and a liquid phase is conventionally explained by the partition coefficient. Partition depends on the polarity of the liquid phase and changes in odorant equilibria have been noted as the liquid phase changes from a purely aqueous environment to an ethanolic solution. However, the dynamics of odorant release rely on physicochemical mechanisms other than partition and, with ethanolic solutions; odorant release is affected by the nature of the gas-liquid interface and the access of ethanol to that interface. Using a range of surface active agents and by using thermal imaging of the surface, the effect of these interfacial effects on odorant release has been probed. The conclusion is that the nature of the interface affects mass transfer in the bulk phase and therefore has the most effect on odorant release prior to consumption.

Introduction

Consumer appreciation of food and beverage flavor depends on sensing a range of chemical stimuli (e.g. taste and smell) and physical stimuli (e.g. viscosity and color). The English language does not offer precise definitions of taste, smell,

In Flavors in Noncarbonated Beverages; Da Costa, N., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

Downloaded by PENNSYLVANIA STATE UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch012

odor, aroma, mouthfeel and flavor. In this paper the word odorant is used to describe compounds that possess odor, smell is defined as the sense of olfaction, the word taste is limited to signals produced by tastants activating the taste buds located on the tongue, mouthfeel is the sensing of the physical stimuli, and flavor is the overall sensation produced by a combination of all signals. Odor is one part of the smell signal and it is sensed by sniffing an alcoholic beverage before consumption, whereas aroma of ethanolic beverages is sensed when the product is consumed. Sniffing, or "nosing" wine to evaluate the "bouquet" is a key part of wine tasting, while the mouth movements that wine tasters use when assessing a wine, are designed to maximize aroma release and amplify the aroma signal reaching the odor receptors. The difference between the definitions of odor and aroma is therefore the route by which the volatile odorant compounds reach the olfactory receptors, located high in the nasal passages. Odor is sensed orthonasally, while aroma is sensed retronasally (*1*). It has been reported that the perception of volatile compounds by the two routes is different (*2*, *3*). This has been explained by the need for humans to use the sense of smell in two different ways. Orthonasal detection senses danger (or food sources) by detecting specific odors (e.g. smoke, predators, ripe fruit odors or malodors associated with rotten food). Signals can be amplified by repeated sniffing which optimizes delivery of the volatile compounds to the odor receptors (*4*). Retronasal detection occurs when food and drink is chewed or swallowed and air from the mouth, containing the volatile compounds, is transferred to the nose. The aroma signals combine with signals from the other senses and produce the overall flavor sensation which is thought to be related to nutritional needs as well as to pleasure centers in the brain.

With alcoholic beverages, ethanol is the major volatile component and concentrations vary between beers (around 3% to 5% v/v) up to spirits (typically around 40% v/v). Since odorants are present at much lower levels (from mg/L to ng/L), ethanol levels in an intermediate ethanol product like wine, exceed odorant levels by factors between 10^5 and 10^{11}. The effect of this excess of ethanol has several effects on sensory perception. First, ethanol creates a stimulus in its own right by activation of trigeminal receptors in the mouth and throat (*5*). Second, ethanol changes the polarity of the liquid phase and causes changes in partition coefficient of many odorants, especially the more hydrophobic compounds (*6*). Third, above certain concentrations, visual features like the "legs" or "tears" of wine form above the surface of the wine due to surface tension effects (*7*) and subsequent evaporation of ethanol (*8*) which leaves the distinctive pattern of tear droplets or legs on the glass surface. In some ways, the multiple effects of ethanol on sensory attributes of alcoholic beverages are akin to the effects of fat on flavor perception in dairy products, where the fat content changes the partition coefficient, contributes to the product's viscosity and may produce a stimulus of its own through an oral fat receptor (*9–13*). In both cases, these multiple effects make it difficult to reduce the fat or ethanol content without seriously affecting the product quality attributes, and this explains the difficulty in making low alcohol versions of popular beverages that deliver the full sensory satisfaction of the original products. While the effect of fat on sensory properties has been widely researched, the role of ethanol on beverage quality has received less attention.

This paper describes some initial work to examine the mechanisms controlling odorant release in ethanolic drinks.

In real life, odor and aroma release occur under semi-controlled conditions in the wine glass and *in vivo*. In the glass, the temperature of the beverage is usually set by the serving temperature recommendation of the supplier and different shaped glasses are used for different beverages (compare a wine glass with a beer tankard) as they are said to optimize the bouquet of that product. When studying the physical chemistry of odorant release from alcoholic beverages, the variation in odor release in a glass, which is open to the atmosphere, is very high due to the random air currents that surround the glass. Measurements made under real life conditions are so variable that they have little practical use. One solution adopted to study odor release from hot beverages (tea) showed the process could be monitored in a consistent way if airflows were controlled by placing a shield around the drinking vessel (*14*) but the methodology is cumbersome and results in an accumulation of odor with time which does not occur naturally. When we consider the situation in the mouth, the average consumer does not control the drinking process or think about aroma release, but expert wine tasters do go through a “chewing” and “gargling” type process which increases the release of wine aroma in the mouth by a factor of around 3 times (unpublished data from our lab). There is also significant interpersonal variation. While there are well-established methods for mimicking odor release, there are few, validated model systems available for *in vivo* release (*15*) and most researchers use human subjects, then use data analysis to reduce the “noise” in the data to uncover trends in release. In this paper, only *in vitro* odor release measurements are used where the conditions can be closely controlled so that the effects of ethanol content and interfacial effects can be clearly measured.

The practical issues in measuring odorant release revolve around suitable methodologies. Headspace measurements can be made under equilibrium conditions from which the partition coefficient can be calculated. Alternatively, a dynamic headspace dilution method can be used to mimic the situation that occurs when a beverage is placed in a glass and the headspace is diluted by currents of air (*16*, *17*). Monitoring the headspace concentrations can be achieved by sampling the air and analyzing serial samples by GC-MS or by one of the direct MS techniques which gives a “real time” readout. In both cases the presence of an excess of ethanol can interfere with the analyses. To overcome this issue with Atmospheric Pressure Chemical Ionization-Mass Spectrometry (APCI-MS), the system was adapted in our laboratory to use ethanol as the charge transfer reagent in place of the usual water and to control the concentration of ethanol in the source to provide consistent and quantitative ionization (*18*, *19*). This analytical method provides a quick and reliable way to monitor odorant release, especially during dynamic headspace analyses where the initial equilibrium concentration falls rapidly as the headspace is diluted with clean gas.

Of the ethanolic beverages, the effect of ethanol on wine properties has been studied for over a hundred years. The “tears of wine” phenomenon was studied in the period 1855-1865 and publications from Thomson (*20*) and Marangoni (*21*) explained the physical basis of this effect. The phenomenon relies on the fact that ethanol is surface active and, in an aqueous solution, congregates at the interface,

Downloaded by PENNSYLVANIA STATE UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch012

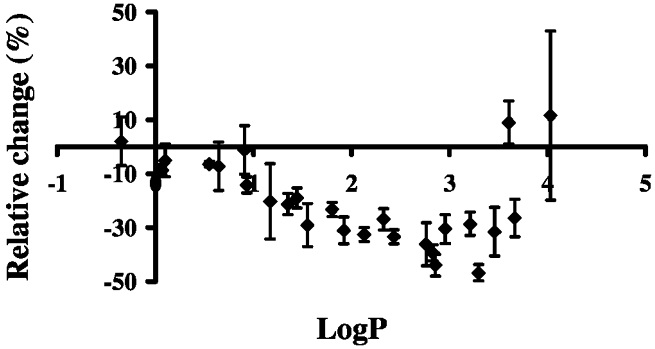


Figure 1. Mean relative change (%) of headspace concentration above a 12% ethanol solution compared to water solutions for twenty-six volatiles plotted against their LogP values. A relative change value of 1 indicates no change, a negative value indicates a decrease in headspace concentration. Error bars are +/-SD.

causing a higher concentration in the surface layer. This reduces surface tension, allowing the surface layer to flow easily and so it creeps up the walls of the glass. However, as this movement occurs, ethanol also evaporates from the surface, which increases the surface tension and the "tears" are due to layers of solutions with different surface tensions which move over one another as described by Marangoni (*21*). Surface evaporation also causes cooling of the interfacial layer which descends through the bulk phase and sets up Rayleigh-Bénard convection which effectively "stirs" the bulk phase. This effect can be visualized if fine gold dust (as used by confectioners) is suspended in a 12% v/v ethanolic solution in a wine glass and air is blown across the solution surface to encourage ethanol evaporation. Clear stirred "cells" can be observed in the bulk phase under these conditions. This is a very different situation to that observed in purely aqueous systems where depletion of odorant at the interface occurs and where mass transfer of odorant from the bulk phase to the interface is the rate limiting step in odorant release (*22*).

Materials and Methods

Absolute ethanol, sodium dodecyl sulfate, Tween 80, Bovine Serum Albumin (BSA), casein, *β*-lactoglobulin, tartaric acid, linoleic acid, sodium hydroxide and the odorants were obtained from Sigma Aldrich (Poole, U.K.). Mannoproteins used in this experiment were purchased from Lallemand Spain (commercial name Optired). According to the technical specifications, the mannoproteins (mannose homopolymers containing protein) were obtained from yeasts inactivated during the exponential growth phase. Remains of proteins and lipids from the cell wall were still present in the product as only a coarse purification was applied. Oak extract was supplied by the Department of Analytical Chemistry, Universidad Zaragoza, Spain.

Downloaded by PENNSYLVANIA STATE UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch012

Downloaded by PENNSYLVANIA STATE UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch012

Static Equilibrium Headspace Measurements

For static headspace studies (*18*), the volatile solution (40mL) was placed in 123mL flasks (Sigma-Aldrich, Poole, U.K.) fitted with a one-port lid. After equilibration for at least two hours at ambient temperature (22°C), headspace was sampled through the port into the APCI-MS with a sample flow rate of 5mL/min. The sample entered the APCI-MS through a heated (140°C) deactivated fused silica transfer line, which minimized any losses or absorption of volatiles. The concentration used for each volatile was within the infinite dilution range to ensure compliance with Henry's Law.

Dynamic HeadSpace (DHS)

Odorant solution (100mL) was placed in glass bottles with 23mL headspace and sealed with screw cap lids. The lids were modified to hold an inlet and outlet tube (3mm id) which carried nitrogen through the headspace at a fixed flow rate (30 or 70mL/min). The bottles were allowed to equilibrate for 2 hr then subjected to DHS.

Monitoring of Headspace

A quadrupole mass spectrometer fitted with an MS Nose interface (LCZ, Micromass, Manchester, U.K.) was used to sample the outflow from the DHS bottles (*23*). Of the 30mL/min outflow, 5mL/min were sampled into the MS Nose. The APCI source was operated with a corona voltage of 4 kV but with a modification to ensure that the final concentration of ethanol in the source was the same whatever the ethanol concentration of the sample. This was achieved by adding ethanol to the nitrogen makeup gas in the range 2.0-11.3μL/L, depending on the ethanol concentration of the sample so that ionization was consistent and quantitative.

Surface Tension Measurements

A tensiometer from SINTERFACE (PAT1, Berlin, Germany) was used. The pendant droplet method measured surface tension of each solution for 180 s (1 point every second). Drop size was 25mm^3 for water and 20mm^3 for the rest of solutions. Density measurements were performed with a DMA 5000 density meter from Anton Paar.

Model Wine Solutions

Oak extract (200 or 1000mg/L) and mannoprotein (16mg/L) were added to solutions containing 4g/L of tartaric acid adjusted to pH 3.6 with 1M NaOH and

12% v/v total ethanol content. Solutions were shaken for 2 hr and then left in contact overnight. All solutions were prepared and measured in triplicate.

Experimental Data on Odor Release from Aqueous and Ethanolic Systems

Effect of Air-Water Partition Coefficient on Odor Release in Ethanolic Systems

The information in the Introduction about the role of ethanol in solution shows that the partition coefficient will be changed, depending on the amount of ethanol present. Figure 1 shows the relative change in equilibrium headspace concentrations between an aqueous and a 12% v/v ethanolic solution for twenty-six odorants selected for their range of hydrophobicities, measured as log P values (*24*). Kaw values for the twenty-six odorants ranged from 10^{-2} to 10^{-5} (calculated with EPIsuite software; www.epa.gov/oppt/exposure/pubs/episuitedl.htm). Odorants considered as hydrophilic (i.e. with a log P less than 1) were not much affected by 12% ethanol but the effect became stronger as log P values increased towards 3. Thereafter, there was some evidence that the trend may reverse around a log P value of 4, although the practical difficulty in measuring these data accurately with compounds that are extremely insoluble in water, made a clear cut conclusion difficult.

What is not clear is whether ethanol will affect odor release under dynamic headspace (DHS) conditions. Previous work (*16*) with purely aqueous solutions showed that odor release was governed by the air water partition coefficients under one set of DHS conditions (Figure 2.).

To investigate the effect of ethanol on the DHS release process, seventeen odorants commonly found in beverage flavorings were added singly to 12% v/v ethanol and subjected to DHS using the same operating conditions as described in Figure 2, Table I shows their release behavior, expressed as the decrease in headspace value from the initial value to the plateau value, and expressed as percentages so that the relative decrease between odorants in water and in 12% v/v ethanol could be seen (*19*).

Inspection of Table I demonstrates that odorant release under DHS conditions is increased in all but two cases (furfuryl alcohol and phenylacetaldehyde) where the factor values show no change in release between water and 12% ethanol. The magnitude of the effect varies from 1 (no effect) to values between 8 to 13 for the poorly water-soluble terpene compounds (e.g. *p*-cymene). This phenomenon is initially counter–intuitive and difficult to explain. Ethanol will act as a solvent for these terpenes and should therefore increase the affinity of the compounds for the liquid phase, not the gas phase and, on the grounds of solubility alone, one would expect the reverse effect. However, given the potential effect of ethanol in assisting mass transfer by “stirring” the bulk phase, the counter argument is that

Downloaded by PENNSYLVANIA STATE UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch012

Downloaded by PENNSYLVANIA STATE UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch012

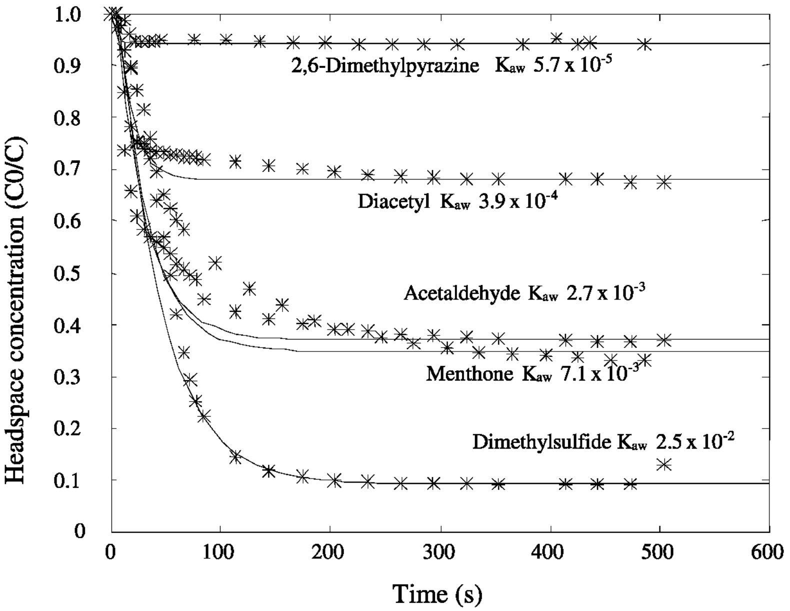


Figure 2. Odor release from aqueous solutions under DHS showing dependence on partition coefficient. The symbols represent the experimental data and the solid lines the general release model proposed (16) change in headspace concentration was expressed as a fraction of the initial concentration C0. Operating conditions for the system were: T=25°C, Surface area= 1 × 10^{-3} m^2, Gas volume= 50 × 10^{-6} m^3, Gas flow=70mL/min.

the ability of these compounds to access the gas-liquid interface accounts for their greater release.

Previous studies (*19*) have also shown that the ethanol effect on odorant release under DHS conditions is dependent on the ethanol concentration and the level at which the effect ceases is compound dependent. The first principle is demonstrated in Figure 3 which shows the release of three odorants from ethanol in the range 0 to 22% v/v ethanol. There is a clear “step” region where the release behavior changes from “water-like” to “ethanol-like”. Similar step profiles are seen for other odorants (*19*).

Evidence that the Bulk Phase Mass Transfer Mechanism Is Correct

The explanation of ethanol being present in excess at the interface and causing an increase in release under DHS conditions by “stirring” of the bulk phase, thus allowing odorants better access to the interfacial layer for partition, is plausible but needed to be tested. One simple experiment was to set up DHS release from water and from ethanol and compare the effect of stirring the water phase to determine if this created the same release behavior from both solutions. Figure 4 shows

Downloaded by PENNSYLVANIA STATE UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch012

Table I. Comparison of odor release under DHS conditions in water and in 12% v/v ethanol. Values in brackets are the percentage coefficient of variation. Change factor is the ratio of the release values

Odorant	*Release from Water*	*Release from Ethanol*	*Change Factor*
Acetaldehyde	27.9 (5)	74.4 (17)	2.67
Propanal	19.5 (24)	82.6 (5)	4.23
Butan-2-ol	69.6 (5)	81.5 (5)	1.17
Diacetyl	71.7 (5)	90.6 (7)	1.26
isoAmyl alcohol	75.9 (2)	89.5 (2)	1.18
Furfuryl alcohol	95.6 (2)	92.6 (2)	0.97
(Z)-3-Hexenol	92.7 (1)	97.5 (1)	1.05
Ethyl 2-butenoate	30.1 (3)	91 (5)	3.02
Ethyl butyrate	13.3 (2)	75.5 (5)	5.68
Phenylacetaldehyde	98.5 (1)	97.8 (0)	0.99
1-Octen-3-one	34.4 (3)	96.6 (1)	2.81
Octanal	8.6 (9)	49.2 (15)	5.72
Ethyl isovalerate	5.5 (13)	52.5 (1)	9.55
p-Cymene	0.9 (33)	11.9 (3)	13.2
Limonene	0.9 (56)	7.9 (10)	8.78
Terpinolene	0.8 (38)	7.0 (11)	8.75
Eucalyptol (1,8-Cineole)	30.1(3)	97.3 (2)	3.23
Linalool	92.2 (1)	96.7 (0)	1.05

the results of such an experiment, carried out with 1-octen-3-one where three replicates of a 12% v/v ethanolic solution gave the release shown by the open square symbols whereas the filled squares show the release from the aqueous solution. When the latter was subjected to stirring at 300 s (Figure 4), the release from the aqueous solution was equivalent to the ethanolic solution, thus providing evidence for the effect.

Another way to test the hypothesis is to observe the change in surface temperature of ethanolic and aqueous solutions during dynamic headspace dilution. Tsachaki *et al.* (*23*) described such an experiment, where an infra red camera recorded higher temperatures at the surface of the 12% v/v ethanolic sample, compared to the aqueous control. The explanation was that, because ethanolic solutions are stirred, the molecular motion brings warmer fluid from the bulk phase to the surface whereas molecular motion in the aqueous sample is limited and therefore the surface undergoes a greater degree of evaporative cooling. From a starting temperature around 21°C, the aqueous sample showed a temperature change of around 1°C within 30 seconds of blowing air across the

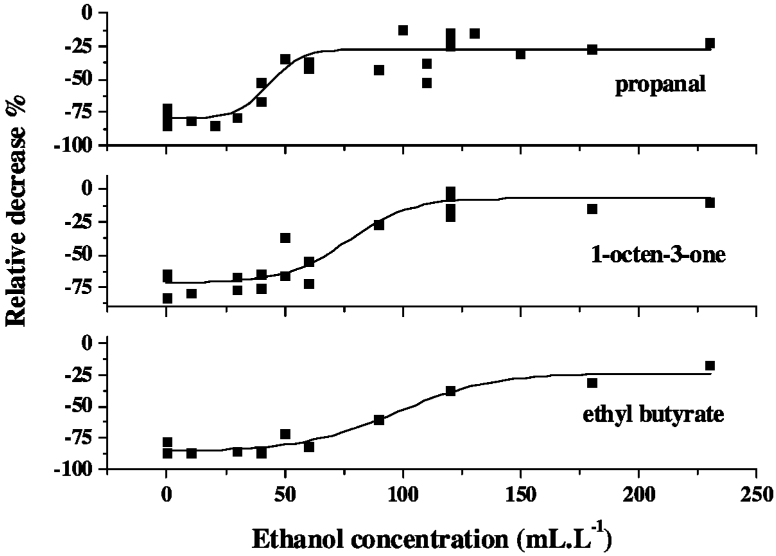


Figure 3. Change in DHS behavior of three odorants as a function of ethanol content, measured as relative decrease in headspace concentration where the equilibrium headspace was 100% and the plateau headspace concentration was measured after 10 min.

surface while the temperature of the ethanolic solutions remained constant at around 21°C.

A third way to examine the hypothesis was to use other surface active agents to disrupt the claimed surface active properties of ethanol on the basis that, without access to the surface layer, ethanolic solutions would show the same release behavior as aqueous solutions. In the presence of low concentrations of sodium dodecyl sulfate (SDS) and Tween 80, the behavior of ethanolic solutions under DHS showed little change, apart from the 1.13 mg/L Tween 80 sample which showed aqueous solution behavior during DHS, despite containing 12% v/v ethanol. At first sight it is difficult to rationalize the results as one might expect all surfactants to behave in the same way at similar concentrations. However, it is necessary to consider the concentration relative to the critical micelle concentrations (CMC) of SDS and Tween 80, not forgetting that the values will be different in ethanolic solutions compared to water. CMC is the minimum concentration needed for a surfactant to form micelles with hydrophobic compounds like oils or odorants with high log P values. The CMC of SDS in 10% v/v ethanol is 5.9mM (*25*) so CMC is equivalent to 1.7g/L and the amounts used in Table II were equivalent to 0.014 and 0.14% of CMC, so micelle formation would not occur at these levels. For Tween, the CMC of Tween 20 in 12% v/v ethanol is 51μM (*26*) and the equivalent in mg/L is 66.8 so the amounts used in Table II represent 1.7 and 0.17% of CMC. Further work to determine the relationship between CMC and changes in DHS need to be carried out.

Downloaded by PENNSYLVANIA STATE UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch012

Downloaded by PENNSYLVANIA STATE UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch012

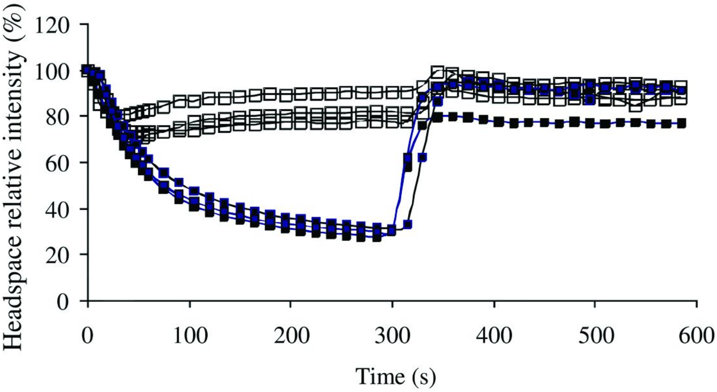


Figure 4. DHS release of 1-octen-3-one from water (filled symbols) and 12% v/v ethanolic solution (open symbols). The three traces are replicates of each treatment. The solutions were left until 300 s, when gentle stirring was applied to the aqueous solution only.

Table II. Changes in DHS headspace concentrations (expressed as percentage change relative to equilibrium value) for three different odorants in ethanolic and aqueous solutions containing different sub-CMC concentrations of SDS or Tween 80

Surfactant	*Concentration (mg/L)*	*1-Octen-3-one*	*Eucalyptol*	*Ethyl 2-butenoate*
Tween® 80	1.13	37 (±3)	39 (±8)	43 (±3)
Tween® 80	0.113	58 (±10)	61 (±14)	62 (±8)
SDS	2.39	88 (±7)	87 (±13)	89 (±7)
SDS	0.239	78 (±14)	78 (±12)	80 (±12)
Control: 12 % ethanol	–	83 (±4)	91 (±3)	84 (±5)
Control: water	–	34 (±3)	30 (±3)	30 (±3)

Proteins are also surface active and a well known food grade protein (*β*-lactoglobulin; extracted from milk) was added to the ethanolic and aqueous solutions of ethyl 2-butenoate which were then subjected to DHS. The traces are shown in Figure 5. The uppermost trace shows the release of ethyl 2-butenoate from 12% v/v ethanolic solution and the shape of the trace shows the typical effect of ethanol, with the headspace concentration showing little change from the equilibrium value as the headspace is diluted. The aqueous control is hidden under the two traces corresponding to the ethanol and aqueous samples containing beta-lactoglobulin. The addition of the protein changes the odorant release so that the ethanol solution with beta-lactoglobulin behaves like the aqueous controls.

Downloaded by PENNSYLVANIA STATE UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch012

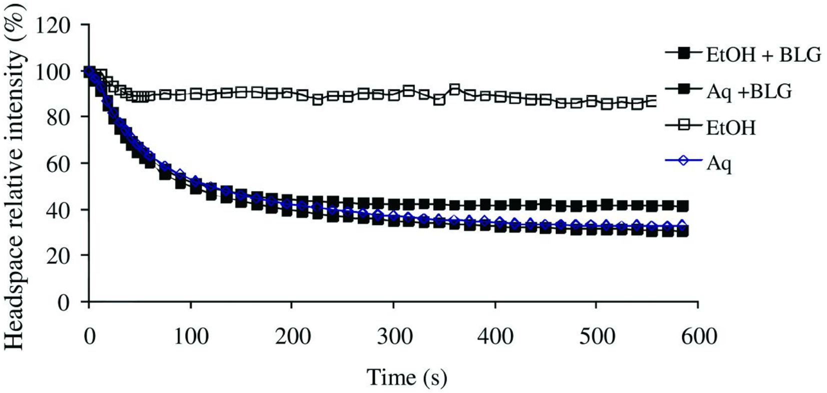


Figure 5. DHS traces from a 12% ethanolic solution (EtOH) and an aqueous (Aq) solution, both containing ethyl2- butenoate. The filled symbols denote samples that also contain beta lactoglobulin (BLG).

Table III. Headspace concentration of odorants in different systems at the end of the DHS process expressed as a percentage of equilibrium headspace concentration. Values are the mean of three replicates (+/-SD)

	1-octen-3-one	*eucalyptol*	*ethyl 2-butenoate*	*p-cymene*
Mannoprotein (16 mg/L) +Oak extract (1000 mg/L) 12% ethanol	33 (±2)	43 (±1)	38 (±2)	4 (±1)
Mannoproteins (16 mg/L) 12% ethanol	35 (±1)	43 (±2)	40 (±2)	4 (±1)
Oak extract (1000 mg/L) 12% ethanol	33 (±1)	44 (±1)	38 (±1)	3 (±0.3)
Control: ethanol 12%	73 (±3)	75 (±2)	73 (±3)	18 (±5)
Control: water	23 (±1)	31 (±1)	31 (±1)	2 (±0.4)

Further experiments were carried out with components of wine which might have surface active properties. The yeast protein, mannoprotein has been reported to affect the aroma intensity of a model wine (*27*) and to change the release of certain aromas during DHS (*28*). An oak extract was also chosen as it contains a mixture of phenol related compounds such as hydrolysable tannins (ellagitannins), aromatic aldehydes (i.e. vanillin), aromatic acids (i.e. gallic acid) and coumarins, some of which exhibit surface activity. In this experiment, citric acid (4g/L) was added to the 12% v/v ethanolic solution and adjusted to pH 3.6 to represent the basic acidity of a wine, so as to test whether pH had an effect on odorant release. Table III shows the results of DHS on four compounds in a range of systems. The aqueous control was depleted to 23% of the original equilibrium headspace value

Table IV. Ethanol concentration and surface tension of a range of solvents and solutions. Values are the mean of three replicates, typical standard deviation ±1 mN/m)

Solution	*Surface tension (mN/m)*	*Ethanol concentration (%)*
Water	72.3	0
12% Ethanol	49.1	12
BSA	48.7	12
Linoleic acid	48.6	12
Model wine	46.6	12
Ethanol	23.0	100

while the 12% v/v ethanol solution was only depleted to 73%. Both oak extract and mannoprotein changed the release behavior of ethanolic solutions such that they were much closer to the water control. There seemed to be no additive effect of oak extract and mannoprotein. However, the concentration of oak extract used (1000mg/L) was in excess of that expected in wines, while mannoprotein levels (16mg/L) were typical for wines. An excess of oak extract was used to determine if there was any effect but, at these levels, a precipitate formed overnight as the samples were stored, prior to analysis. The solutions were analyzed with the precipitate present. Although the presence of the precipitate suggested a decrease in the effective mannoprotein and polyphenol concentrations, this was not evident from the experimental data. A possible explanation for this fact is that all mannoprotein was bound and precipitated, and the depletion observed was solely due to the excess oak extract. To test this hypothesis, a second experiment was carried out using 200mg/L oak extract (data not shown) and the results were very similar to those presented in Table III. Thus it appears that both mannoprotein and oak extract do exert some effect on odorant release.

Measurement of Surface Tension

To provide further information on the effect of surface active agents at the interface, the surface tension of several systems was measured (Table IV.) where the high surface tension of water (72.3mN/m) compared to ethanol (23mN/m) can be seen. The surface tension values for 12% v/v ethanol (with or without BSA or linoleic acid) and model wine solution were very similar and further work is required to establish whether the pendant drop method mimics the situation in the DHS experiments.

Conclusion

The ability to measure odorant release reliably in the presence of ethanol has allowed the experiments described above to be carried out. The results show

Downloaded by PENNSYLVANIA STATE UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch012

how ethanol affects odorant release and confirms the role of ethanol in decreasing the air-liquid partition coefficient although the increased release during dynamic headspace dilution was unexpected. Using data from the mid 1800s (Thomson and Marangoni), the increased release during dynamic headspace dilution can be explained. The hypothesis that ethanol at the interface creates a “stirring” effect in the bulk phase due to the “Marangoni sequence of events” has been tested in several ways. Physically stirring an aqueous phase shows odorant release comparable with unstirred ethanol solutions. Adding surface active agents at very low concentration does disrupt the interfacial effects and, combined with the thermal imaging data, reinforces the evidence.

It should be noted that this process only occurs when there is a bulk phase involved. It therefore applies to alcoholic drinks prior to consumption but is unlikely to affect odorant release during drinking as release in mouth occurs from thin films and over very short time scales.

Downloaded by PENNSYLVANIA STATE UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch012

Acknowledgments

RL thanks Programa Europa XXI (DGA CAI) for funding a fellowship to work at the University of Nottingham and MT is grateful to the Greek State Scholarship Foundation (IKY) for a PhD scholarship.

References

1. Taylor, A. J. Volatile flavor release from foods during eating. *Crit. Rev. Food Sci. Nutr.* **1996**, *36* (8), 765–784.
2. Hummel, T.; Heilman, S.; Landis, B. N.; Redem, J.; Frasnelli, J.; Small, D.; Gerber, J. Perceptual differences between chemical stimuli presented through the ortho- and retronasal route. *Flavor Frag. J.* **2006**, *21*, 42–47.
3. Diaz, M. E. Comparison between orthonasal and retronasal flavour perception at different concentrations. *Flavor Frag. J.* **2004**, *19* (6), 499–504.
4. Laing, D. G. The characteristics of human sniffing behavior that provide optimum perception of odors. *Chem. Senses* **1985**, *10* (3), 412.
5. Rothe, M.; Schrodter, M. Flavour Contribution of Ethanol: A Neglected Aroma Compound. In *Flavour Science: Recent Developments*; Taylor, A. J., Mottram, D. S., Eds.; Royal Society of Chemistry: U.K., 1996.
6. Conner, J. M.; Birkmyre, L.; Paterson, A.; Piggott, J. R. Headspace concentrations of ethyl esters at different alcoholic strengths. *J. Sci. Food Agric.* **1998**, *77*, 121–126.
7. Fournier, J. B.; Cazabat, A. M. Tears of wine. *Europhys. Lett.* **1992**, *20*, 517–522.
8. Walstra, P. *Physical Chemistry of Foods*; CRC Press: Boca Raton, FL, 2003; p 807.

Downloaded by PENNSYLVANIA STATE UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch012

9. Barylkopikielna, N.; Martin, A.; Mela, D. J. Perception of taste and viscosity of oil-in-water and water-in-oil emulsions. *J. Food Sci.* **1994**, *59* (6), 1318–1321.
10. Brauss, M.; Cayeux, I.; Harvey, B. A.; Linforth, R. S. T.; Taylor, A. J. COST96 Interactions of Food Matrix with Small Ligands. In *The Effect of Changing the Fat Content on Flavour Release in a Simple Food System*; Guichard, E., Ed.; EU: Athens, 1998; pp 117−122.
11. de Araujo, I. E.; Rolls, E. T. Representation in the human brain of food texture and oral fat. *J. Neurosci.* **2004**, *24* (12), 3086–3093.
12. Gilbertson, T. A. Gustatory mechanisms for the detection of fat. *Curr. Opin. Neurobiol.* **1998**, *8* (4), 447–452.
13. Hort, J.; Cook, D. J. Formulating Low Fat Foods: The Challenge of Retaining Flavour Quality. In *Modifying Flavour in Food*; Taylor, A. J., Hort, J., Eds.; Woodhead: Cambridge, 2007; pp 131−145.
14. Wright, J.; Wulfert, F.; Hort, J.; Taylor, A. J. Effect of preparation conditions on release of selected volatiles in tea headspace. *J. Agric. Food Chem.* **2007**, *55*, 1445–1453.
15. Linforth, R. S. T. Modelling Flavour Release. In *Food Flavour Technology*; Taylor, A. J., Ed.; Sheffield Academic Press: Sheffield, 2002; pp 185−209.
16. Marin, M.; Baek, I.; Taylor, A. J. Flavour release from aqueous solutions under dynamic headspace dilution conditions. *J. Agric. Food Chem.* **1999**, *47*, 4750–4755.
17. Reineccius, G. A. *Flavor Chemistry and Technology*, 2nd ed.; Taylor & Francis: Boca Raton, FL, 2006; p 489.
18. Aznar, M.; Tsachaki, M.; Linforth, R. S. T.; Ferreira, V.; Taylor, A. J. Headspace analysis of volatile organic compounds from ethanolic systems by direct APCI-MS. *Int. J. Mass Spec.* **2004**, *239*, 17–25.
19. Tsachaki, M.; Linforth, R. S. T.; Taylor, A. J. Dynamic headspace analysis of the release of volatile organic compounds from ethanolic systems by direct APCI-MS. *J. Agric. Food Chem.* **2005**, *53*, 8328–8333.
20. Thomson, J. On certain curious motions observable on the surfaces of wine and other alcoholic liquours. *Philos. Mag.* **1855**, *10*, 330.
21. Marangoni, C. On the Expansion of a Drop of Liquid Floating on the Surface of Another Liquid. University of Pavia: Pavia, Italy, 1865.
22. Bakker, J.; Boudaud, N.; Harrison, M. Dynamic release of diacetyl from liquid gelatin in the headspace. *J. Agric. Food Chem.* **1998**, *46* (7), 2714–2720.
23. Tsachaki, M.; Gady, A.-L.; Kalopesas, M.; Athes, V.; Linforth, R. S. T.; Marin, M.; Taylor, A. J. Effect of ethanol, temperature and gas flow rate on volatile release from aqueous solutions under dynamic headspace dilution conditions. *J. Agric. Food Chem.* **2008**, *56*, 5308–5315.
24. Tsachaki, M.; Aznar, M.; Linforth, R. S. T.; Taylor, A. J. Dynamics of Flavour Release from Ethanolic Solutions. In *Flavour Science: Recent Advances and Trends*; Bredie, W. L. P., Petersen, M. A., Eds.; Elsevier: Amsterdam, 2006; Vol. 43, pp 441−444.
25. Javadian, S.; Gharibi, H.; Sohrabi, B.; Bijanzadeh, H.; Safarpour, M. A.; Behjatmanesh-Ardakani, R. Determination of the physico-chemical

parameters and aggregation number of surfactant in micelles in binary alcohol-water mixtures. *J. Mol. Liq.* **2008**, *137* (1-3), 74–79.

26. Nino, R. R.; Patino, J. M. R. Surface tension of bovine serum albumin and Tween 20 at the air−aqueous interface. *J. Am. Oil Chem. Soc.* **1998**, *75* (10), 1241–1248.
27. Lubbers, S.; Voilley, A.; Feuillat, M.; Charpentier, C. Influence of mannoproteins from yeast on the aroma intensity of a model wine. *Food Sci. Technol.-Lebensm.-Wiss. Technol.* **1994**, *27* (2), 108–114.
28. Chalier, P.; Angot, B.; Delteil, D.; Doco, T.; Gunata, Z. Interactions between aroma compounds and whole mannoprotein isolated from Saccharomyces cerevisiae strains. *Food Chem.* **2007**, *100* (1), 22–30.

Downloaded by PENNSYLVANIA STATE UNIV on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch012

Chapter 13

Analysis of Volatiles in Limoncello Liqueur and Aging Study with Sensory

Neil C. Da Costa*,[1] **and Theodore J. Anastasiou**[2]

[1]**International Flavors & Fragrances Inc., 1515 Highway 36, Union Beach, NJ 07735, USA**
[2]**12 Ladwood Drive, Holmdel, NJ 07733, USA**
***neil.dacosta@iff.com**

Downloaded by STANFORD UNIV GREEN LIBR on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch013

Limoncello is a liqueur originating from Southern Italy, and in more recent times has also been produced in neighboring countries around the Mediterranean region. It is traditionally produced by the maceration (*1*) of lemon peel in grain alcohol, water and sugar and has a distinctive bright yellow color. It has a sweet lemony taste which is not sour, as it does not contain any lemon juice. This paper describes its homemade preparation, liquid/liquid solvent extraction and gas chromatography (GC) analysis. In addition detailed gas chromatography-mass spectrometry (GC-MS) analytical data was obtained on a sector instrument of fresh limoncello versus five month aged Limoncello volatiles. Previous analytical work (*2*, *3*) has described over sixty volatile components in Limoncello; the analytical work presented here identifies over two hundred components. In addition compositional changes that occur with aging are described and related to gas-chromatography-olfactometry (GC-O) work. These changes appear to differ from those found in lemon juices or carbonated drinks.

Introduction

Limoncello is a lemon-based liqueur produced predominantly in Sorrento in southern Italy, and also in neighboring countries in the Mediterranean region. It is

In Flavors in Noncarbonated Beverages; Da Costa, N., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

consumed as an aperitif or as a digestive. Traditionally, Limoncello is made from four ingredients: lemon peel (without pith), grain alcohol, water and sugar. It has a sweet lemony taste which is not sour since it does not contain any lemon juice. Limoncello is a syrupy, opaque emulsion which has a distinctive bright yellow color.

The standard way to make Limoncello is to first extract the lemon peels with grain alcohol. This process takes several weeks and results in a highly colored extract. The extraction is very thorough at removing the oils from the rinds as indicated by the peels turning from pliable and yellow to brittle and colorless. Following the extraction a simple syrup is added to the extract causing instant emulsification. This mixture is allowed to “marry” for several additional weeks. It can be stored long term at room temperature, but must be transferred to the freezer before serving.

While Limoncello was traditionally made in the home, several commercial brands exist in the U.S. market. This number has been increasing steadily in recent years with offerings from countries other than Italy, containing citrus fruits other than lemons (e.g. oranges).

Keeping Limoncello frozen maintains it at a serving temperature and also minimizes changes in the chemical composition due to reactions of the extracted oils with water and ethanol, as well as with air and light. It is well known that during the aging of citrus based beverages, several compositional changes occur such as the rearrangement and reduction of citral (neral and geranial) to such components as p-cymene, p-cymen-8-ol, p-methylacetophenone and p-α-dimethylstyrene (*4*). Store-bought Limoncellos are kept at ambient temperatures or higher for extended times during shipping, warehousing, and on store shelves. There is a higher likelihood of commercially available Limoncellos having less of a true flavor profile than their homemade counterparts. Thus it is the aim of this investigation to determine the compositional changes that occur during Limoncello aging.

Several examples exist in the literature (*5–10*) on the compositional analysis of Limoncello, mainly to establish authenticity and detect adulteration. The most recent reports from Versai *et a*l (*2*) and Crupi *et al* (*3*), while very thorough in their analyses, only detail a limited number of volatile compounds, thirty-one and sixty-three volatiles, respectively. Additionally no literature to date explores the effect of aging on the composition. Herein we report the identification of over two hundred volatile compounds and the observed compositional changes that occur during aging. Since it is difficult to obtain “fresh” samples of the commercial products we made and analyzed our own Limoncello to be able to accurately track these changes.

Downloaded by STANFORD UNIV GREEN LIBR on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch013

Downloaded by STANFORD UNIV GREEN LIBR on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch013

Table I. Volatile Composition of the Ethanolic Lemon Peel Extract

	Component	*Conc.*[a] *FID %*	*Kovats*[b]	*Confirmation*
1	Acetic acid	0.68	550	RT[c], MS[d]
2	Furfural	0.06	800	RT, MS
3	Furfuryl alcohol	0.26	833	RT, MS
4	isoValeric acid	0.11	835	RT, MS
5	3-Methyl-3-butenoic acid	0.52	855	RT, MS
6	Heptanal	0.22	878	RT, MS
7	2-Hydroxy-2-cyclopentenone	0.12	892	RT, MS
8	*α*-Thujene	0.57	923	RT, MS
9	*α*-Pinene	2.36	931	RT, MS
10	Camphene	0.44	943	RT, MS
11	4-Hydroxyhexan-2,3,5-trione	0.06	951	MS
12	6-Methyl-5-hepten-2-one	0.05	963	RT, MS
13	*β-Pinene*	22.23	971	RT, MS
14	Octanal	0.03	980	RT, MS
15	*β*-Myrcene	1.22	981	RT, MS
16	*α*-Phellandrene	0.09	996	RT, MS
17	*α*-Terpinene	0.39	1003	RT, MS
18	p-Cymene	0.02	1011	RT, MS
19	Limonene	49.81	1023	RT, MS
20	(*Z*)-β-Ocimene	0.09	1028	RT, MS
21	(*E*)-β-Ocimene	0.24	1038	RT, MS
22	*γ*-Terpinene	11.35	1051	RT, MS
23	(*E*)-Sabinene hydrate	0.09	1055	RT, MS
24	Terpinolene	0.53	1076	RT, MS
25	(*Z*)-Sabinene hydrate	0.29	1083	RT, MS
26	Linalool	0.14	1085	RT, MS
27	5-Hydroxy-5,6-dihydromaltol	0.67	1102	MS
28	Camphor	0.09	1114	RT, MS
29	Citronellal	0.16	1132	RT, MS
30	Benzoic acid	0.13	1160	RT, MS
31	Terpinen-4-ol	0.06	1164	RT, MS
32	*α*-Terpineol	0.36	1168	RT, MS
33	5-Hydroxymethylfurfural	0.27	1186	RT, MS
34	Decanal	0.07	1188	RT, MS
35	4-Vinylphenol	0.26	1195	RT, MS
36	Nerol	0.24	1211	RT, MS
37	Neral	0.52	1216	RT, MS
38	Geraniol	0.41	1236	RT, MS
39	Geranial	0.83	1244	RT, MS
40	2-Methoxy-4-vinylphenol	0.28	1286	RT, MS
41	Undecanal	0.05	1287	RT, MS
42	Nonyl acetate	0.38	1297	RT, MS

Continued on next page.

Table I. (Continued). Volatile Composition of the Ethanolic Lemon Peel Extract

	Component	*Conc.*[a] *FID %*	*Kovats*[b]	*Confirmation*
43	*β*-Citronellyl acetate	0.08	1334	RT, MS
44	Neryl acetate	0.38	1343	RT, MS
45	Geranyl acetate	0.54	1360	RT, MS
46	Limonen-10-yl acetate	0.09	1388	RT, MS
47	(*Z*)-*α*-Bergamotene	0.40	1419	RT, MS
48	*β*-Caryophyllene	0.31	1422	RT, MS
49	(*E*)-*α*-Bergamotene	0.40	1435	RT, MS
50	(*E*)-*β*-Farnesene	0.07	1453	RT, MS
51	*α*-Humulene	0.01	1455	RT, MS
52	Geranyl propionate	0.05	1468	RT, MS
53	Valencene	0.17	1492	RT, MS
54	Bicyclogermacrene	0.14	1494	RT, MS
55	(*Z*)-*α*-Bisabolene	0.07	1496	RT, MS
56	*β*-Bisabolene	0.56	1504	RT, MS
57	2.6-dimethoxy-4-vinylphenol	0.12	1545	RT, MS
58	*α*-Bisabolol	0.07	1671	RT, MS

[a] Concentrations given were determined by GC-FID adjusted using an internal standard and applied correction factors. [b] Kovats = Standard retention data using an alkane homologous series on non-polar column. [c] RT = Retention time on 50m OV1 column. [d] MS = Mass spectrum (EI 70eV).

Downloaded by STANFORD UNIV GREEN LIBR on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch013

Materials and Methods

Limoncello Preparation

Limoncello was prepared as follows according to the Washington Post article (*11*). Seventeen large store-bought organic lemons (~175g each, ~3000g total) were washed, dried, and carefully peeled as to minimize the amount of pith. The peels were submerged in 1500mL of grain alcohol (95% abv, 190 proof) ("Everclear", Luxco, Inc., St. Louis, MO, USA). This mixture was allowed to stand at room temperature for 14 days with brief stirring every other day. After 14 days the spent lemon peels were removed by filtration leaving a dark yellow solution. 1700g of a simple syrup solution consisting of equal parts (1350g) water and granulated sugar were then added to the extract, resulting in an opaque yellow emulsion. This emulsion was allowed to sit at room temperature for an additional 21 days, again with stirring every other day. At the end of the 21 day period the Limoncello was bottled and stored either in the freezer (-18°C) or at room temperature. The approximate alcohol concentration was calculated to be 50% (100 proof). When stored in the freezer the Limoncello further emulsifies and thickens. At room temperature the emulsion is very stable, with no separation occurring even after five months.

Liquid/Liquid Solvent Extraction

260mL of freshly refrigerated Limoncello was added to 250mL of distilled water in a 1L glass separator and twice extracted with 100mL of dichloromethane (Aldrich, St. Louis, MO). Chilled centrifugation (3000rpm for 10 mins) was required to aid separation of the phases due to emulsion formation. The two combined extracts were back extracted with 100mL of distilled water in a separator, to reduce sugar content, dried over anhydrous sodium sulfate (Aldrich), filtered and concentrated to 1mL using a Zymark turbovaporator. Similarly 260mL of five month old Limoncello stored at room temperature, was extracted as previously described. Diethyl phthalate was used as an internal standard dosed at 0.01%.

Downloaded by STANFORD UNIV GREEN LIBR on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch013

Gas Chromatography Analysis (GC)

The liquid/liquid extract was analyzed using an HP6890 gas chromatograph with split/splitless injection and a flame ionization detector (Hewlett Packard, Wilmington, PA). The extract was injected onto an OV1 capillary column (50m × 0.32mm i.d., 0.5µm film thickness, Restek, Bellefonte, PA) in the split (split ratio 40:1) and splitless mode. Carrier gas was helium with a flow rate of 1.0mL/min. Injection port temperature was 250°C and the detector temperature was 320°C. Column temperature was programmed from 40°C to 270°C at a rate of 2°C/min with a holding time at 270°C of 10 minutes. The extract was also injected onto an HP5890 gas chromatograph with split/splitless injection and a flame ionization detector (FID) fitted with a Carbowax capillary column (50m × 0.32mm i.d., 0.3µm film thickness, Restek, Bellefonte, PA) using the same injection and detection techniques. The GC oven was run using temperature programming with an initial temperature of 60°C held for 10 minutes, ramped at 2°C/min to a final temperature of 220°C and held for 20 minutes. To aid in the detection of sulfur containing compounds, the extract was analyzed by HP6890 gas chromatograph equipped with an Antek chemiluminescence detector (Hewlett Packard, Wilmington, PA). The column was an OV1 capillary column and the analysis was conducted in splitless mode. Injection and detection temperatures were as previously described with the following temperature program 35-290°C @ 2°C/min. All data was collected and stored by using HP ChemStation software (Hewlett Packard, Wilmington, PA).

Gas Chromatography-Mass Spectrometry Analysis (GC-MS)

Identification of components in the extracts was conducted by mass spectrometry. The sample was injected onto an HP6890 GC. The chromatographic conditions for the OV-1 column were the same as described for GC analysis. The end of the GC capillary column was inserted directly into the ion source of the mass spectrometer via a heated transfer line maintained at 280°C. The mass spectrometer was a Micromass Autospec high resolution, double-focusing,

In Flavors in Noncarbonated Beverages; Da Costa, N., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

Downloaded by STANFORD UNIV GREEN LIBR on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch013

magnetic sector instrument. The mass spectrometer was operated in the electron ionization mode (EI), scanning from m/z 450 to m/z 33 @ 0.3 seconds per decade. For analysis on the Carbowax phase (50m × 0.32mm i.d., 0.3µm film thickness Carbowax capillary column), the sample was introduced via an HP5890 GC into a Micromass Autospec mass spectrometer. GC oven conditions were the same as outlined above. The mass spectrometer was operated in EI mode scanning from m/z 450 to m/z 33 @ 0.3 seconds per decade. Spectra obtained from both phases were analyzed using the MassLib data system referencing an IFF in-house library and the commercial Wiley 8, NIST 98 and other libraries. The identification of flavor components was confirmed by interpretation of MS data and by GC relative retention indices based on a calibration with alkanes.

Gas Chromatography-Olfactometry Analysis (GC-O)

For the gas chromatography-olfactometry sessions, the extract was injected onto an HP6890 gas chromatograph equipped with an FID and odor port Model ODP-2 (Gerstel, Inc., Baltimore, MD). The FID: Odor port split was 1:6. The chromatographic conditions were the same as described previously. Three trained panelists smelled through each of the two extracts twice and recorded their aroma descriptors (Table III). From these it was possible to differentiate subtle aroma differences between the samples and produce sensory spidergraphs for fresh and aged Limoncello (Figure 4).

Results and Discussion

Ethanolic Lemon Peel Extract Analysis

This extract was produced after filtering off lemon peels (*12–14*) and before the addition of sugar syrup. It was analyzed as a neat ethanolic extract via GC and GC-MS, and results are given in Table I. As expected limonene (50%) was the most prevalent compound, comprising half of the volatiles. β-Pinene (22%) and γ-terpinene (11%) were also major components and to a lesser extent α-pinene (2%) and β-myrcene (1%). The extract also shows a low concentration of sugar degradation present in the grain alcohol.

Limoncello Extract Analysis

Detailed GC-MS analysis of the fresh, chilled and five month aged, ambient, Limoncello respectively, was conducted (Table II). The GC profiles of both extracts were similar with small concentration and compositional changes. Figure 1 shows a typical aged Limoncello chromatogram.

Downloaded by STANFORD UNIV GREEN LIBR on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch013

Table II. Volatile Composition of Fresh and Aged Limoncello[a]

	Component	*Fresh FID%*	*Aged FID%*	*Kovats*	*Confirmation[b]*
1	Acetaldehyde	tr[c]	tr	427	RT[d], MS[e]
2	3-Methyl-1-butene	tr	tr	450	RT, MS
3	Acetone	tr	tr	475	RT, MS
4	Isoprene	tr	tr	490	RT, MS
5	2-Methyl-2-propenal	tr	tr	515	RT, MS
6	Acetic acid	tr	tr	550	RT, MS
7	3-Buten-2-one	tr	tr	575	RT, MS
8	Butan-2-one	tr	tr	578	RT, MS
9	Prenol	tr	tr	590	RT, MS
10	Ethyl acetate	tr	tr	594	RT, MS
11	2-Methylbutan-2-ol	tr	tr	603	RT, MS
12	3-Methylbutan-2-one	tr	tr	612	RT, MS
13	Valeraldehyde	tr	tr	672	RT, MS
14	Pentan-2-ol	tr	tr	683	RT, MS
15	Acetaldehyde diethyl acetal	0.02	0.05	722	RT, MS
16	Acetaldehyde butan-2,3-diol acetal	tr	tr	734	RT, MS
17	Toluene	tr	tr	750	RT, MS
18	1-Methyl-1,3-cyclohexadiene	tr	tr	755	MS
19	Butyric acid	tr	tr	770	RT, MS
20	Ethyl pyruvate	tr	tr	775	RT, MS
21	Hexanal	tr	tr	776	RT, MS
22	Hexan-2-ol	tr	tr	781	RT, MS
23	Furfural	tr	tr	800	RT, MS
24	Octane	tr	tr	800	RT, MS
25	Ethyl 3-methyl-1-butenyl ether	tr	tr	825	RT, MS
26	isoValeric acid	tr	tr	835	RT, MS
27	(Z)-3-Hexenol	tr	tr	838	RT, MS
28	(*E*)-2-Hexenol	tr	tr	850	RT, MS
29	Hexanol	tr	tr	854	RT, MS
30	isoAmyl acetate	tr	tr	857	RT, MS
31	2-Methylbutyl acetate	tr	tr	860	RT, MS
32	Styrene	tr	tr	872	RT, MS
33	Ethyl valerate	tr	tr	883	RT, MS
34	Tricyclene	tr	tr	920	RT, MS
35	α-Thujene	0.44	0.26	923	RT, MS
36	α-Pinene	1.92	1.09	931	RT, MS
37	Benzaldehye	tr	tr	931	RT, MS
38	2,4(10)-Thujadiene	tr	tr	935	MS
39	α-Fenchene	tr	tr	941	RT, MS
40	Camphene	0.08	0.04	943	RT, MS
41	Diethyl oxalate	tr	tr	950	RT, MS
42	3-Methyl-3-cyclohexenone	tr	tr	953	MS

Continued on next page.

Downloaded by STANFORD UNIV GREEN LIBR on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch013

Table II. (Continued). Volatile Composition of Fresh and Aged Limoncello[a]

	Component	*Fresh FID%*	*Aged FID%*	*Kovats*	*Confirmation[b]*
43	Hexanoic acid	tr	tr	963	RT, MS
44	6-Methyl-5-hepten-2-one	tr	tr	963	RT, MS
45	Sabinene	2.29	0.74	965	RT, MS
46	*β*-Pinene	19.13	6.68	971	RT, MS
47	Octanal	0.03	0.01	980	RT, MS
48	*β*-Myrcene	1.20	1.71	981	RT, MS
49	Valeraldehyde diethyl acetal	tr	tr	984	RT, MS
50	pseudo-Limonene	0.01	tr	995	RT, MS
51	*α*-Phellandrene	0.02	0.03	996	RT, MS
52	Decane	tr	tr	1000	RT, MS
53	*σ*-Car-3-ene	tr	tr	1004	RT, MS
54	Phenylacetaldehyde	tr	tr	1010	RT, MS
55	*α*-Terpinene	0.05	0.04	1003	RT, MS
56	p-Cymene	0.36	0.83	1011	RT, MS
57	*β*-Phellandrene	tr	tr	1021	RT, MS
58	1,8-Cineole	tr	tr	1021	RT, MS
59	Limonene	54.05	52.47	1023	RT, MS
60	(*Z*)-*β*-Ocimene	0.70	0.60	1028	RT, MS
61	Acetophenone	tr	tr	1035	RT, MS
62	Melonal	tr	tr	1036	RT, MS
63	(*E*)-*β*-Ocimene	0.13	0.08	1038	RT, MS
64	*γ*-Terpinene	12.22	8.09	1051	RT, MS
65	(*E*)-Sabinene hydrate	0.20	0.10	1055	RT, MS
66	Octanol	tr	tr	1055	RT, MS
67	Fenchone	tr	tr	1068	RT, MS
68	p-*α*-Dimethylstyrene	tr	tr	1074	RT, MS
69	Terpinolene	0.24	0.27	1076	RT, MS
70	Rosefuran	tr	tr	1079	RT, MS
71	Nonanal	0.13	tr	1083	RT, MS
72	(*Z*)-Sabinene hydrate	0.13	0.18	1083	RT, MS
73	Linalool	0.32	0.19	1085	RT, MS
74	(*Z*)-4,8-Dimethyl-1,3,7-nonatriene	tr	tr	1089	RT, MS
75	Heptyl acetate	tr	tr	1094	RT, MS
76	p-Mentha-1,5,8-triene	tr	tr	1097	RT, MS
77	Fenchol	tr	tr	1099	RT, MS
78	Undecane	tr	tr	1105	RT, MS
79	(*Z*)-p-Mentha-2,8-dienol	0.03	0.02	1107	RT, MS
80	Pinanol	tr	tr	1109	RT, MS
81	(*Z*)-p-Menth-2-enol	0.01	0.01	1110	RT, MS
82	(*E*)-4,8-Dimethyl-1,3,7-nonatriene	tr	tr	1112	RT, MS
83	Camphor	0.02	0.02	1114	RT, MS
84	(*E*)-p-Mentha-2,8-dienol	0.01	0.01	1115	RT, MS

Continued on next page.

Downloaded by STANFORD UNIV GREEN LIBR on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch013

Table II. (Continued). Volatile Composition of Fresh and Aged Limoncello[a]

	Component	*Fresh FID%*	*Aged FID%*	*Kovats*	*Confirmation[b]*
85	Limonene-1,2-epoxide i	tr	tr	1117	RT, MS
86	Limonene-1,2-epoxide ii	tr	tr	1121	RT, MS
87	(*E*)-p-Menth-2-enol	0.03	0.03	1126	RT, MS
88	(*E*)-Sabinene hydrate ethyl ether	nd[f]	0.08	1129	RT, MS
89	isoPulegol i	tr	tr	1131	RT, MS
90	Citronellal	0.09	0.03	1132	RT, MS
91	6-Methylbicyclo[3.3.0]oct-2-en-7-one	tr	tr	1133	MS
92	isoPulegol ii	tr	tr	1135	RT, MS
93	2-Me-6-methylene-1,7-octadien-3-ol	tr	tr	1137	RT, MS
94	isoCitral	0.01	0.01	1140	RT, MS
95	Ethyl hydrogen succinate	tr	tr	1145	RT, MS
96	Borneol	0.01	0.01	1151	RT, MS
97	Nonanol	0.02	nd	1160	RT, MS
98	(*Z*)-Sabinene hydrate ethyl ether	nd	0.09	1161	RT, MS
99	Octanoic acid	tr	tr	1161	RT, MS
100	Limonen-4-ol	0.03	0.37	1163	RT, MS
101	p-Cymen-8-ol	0.02	tr	1163	RT, MS
102	Terpinen-4-ol	0.14	0.02	1164	RT, MS
103	*σ*-Terpineol	tr	tr	1168	RT, MS
104	Verbenone	tr	tr	1172	RT, MS
105	(*E*)-p-Mentha-1(7),8-dien-2-ol	tr	tr	1172	RT, MS
106	*α*-Terpineol	0.46	0.36	1177	RT, MS
107	p-Menth-2-enyl ethyl ether	nd	0.01	1181	RT, MS
108	Myrtenol	tr	tr	1181	RT, MS
109	4,7-Dime-bicyclo[3.2.1]oct-3-en-6-one	tr	tr	1184	MS
110	(*Z*)-Piperitol	tr	tr	1188	RT, MS
111	Decanal	0.04	0.02	1188	RT, MS
112	Benzothiazole	tr	tr	1190	RT, MS
113	Sabinol	0.01	nd	1191	RT, MS
114	Octyl acetate	tr	tr	1193	RT, MS
115	(*E*)-Carveol	nd	0.02	1198	RT, MS
116	2,3-Epoxygeranial i	0.04	0.02	1200	RT, MS
117	Dodecane	tr	tr	1200	RT, MS
118	(Z)-p-Mentha-1(7),8-dien-2-ol	tr	tr	1207	RT, MS
119	2,3-Epoxygeranial ii	0.03	0.01	1209	RT, MS
120	Terpinen-4-yl ethyl ether	tr	tr	1209	RT, MS
121	Nerol	tr	tr	1211	RT, MS
122	Ascaridole	tr	tr	1211	RT, MS
123	Neral	0.79	0.49	1216	RT, MS
124	Piperitone	0.02	0.03	1225	RT, MS
125	Geraniol	0.19	0.31	1236	RT, MS
126	isoPiperitenone	tr	tr	1241	RT, MS

Continued on next page.

Downloaded by STANFORD UNIV GREEN LIBR on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch013

Table II. (Continued). Volatile Composition of Fresh and Aged Limoncello[a]

	Component	*Fresh FID%*	*Aged FID%*	*Kovats*	*Confirmation*[b]
127	Geranial	1.05	0.57	1244	RT, MS
128	Perilla aldehyde	tr	tr	1245	RT, MS
129	*α*-Terpinyl ethyl ether	0.01	0.04	1249	RT, MS
130	Nonanoic acid	tr	tr	1253	RT, MS
131	Thymol	0.05	0.01	1264	RT, MS
132	Limonen-10-ol	tr	tr	1268	RT, MS
133	Bornyl acetate	0.03	0.02	1271	RT, MS
134	Ascardole epoxide	0.06	0.03	1280	RT, MS
135	Octanal diethyl acetal	tr	tr	1284	RT, MS
136	Carvacrol	0.05	0.01	1284	RT, MS
137	Undecanal	tr	tr	1287	RT, MS
138	Nonyl acetate	tr	tr	1297	RT, MS
139	Tridecane	tr	tr	1300	RT, MS
140	Methyl geranate	tr	tr	1303	RT, MS
141	(*E*)-Terpin hydrate	tr	tr	1303	RT, MS
142	Limonen-1,2-diol	tr	tr	1309	RT, MS
143	Limonen-4-yl hydroperoxide	tr	tr	1314	RT, MS
144	(*E*)-Carvyl acetate	tr	tr	1318	RT, MS
145	Geranic acid	tr	tr	1330	RT, MS
146	*α*-Terpinyl acetate	tr	tr	1334	RT, MS
147	*β*-Citronellyl acetate	tr	tr	1334	RT, MS
148	Neryl acetate	0.39	0.54	1343	RT, MS
149	*α*-Cubebene	tr	tr	1350	RT, MS
150	Vanillin	tr	tr	1358	RT, MS
151	*γ*-Terpinyl acetate	tr	tr	1359	RT, MS
152	Geranyl acetate	0.50	0.30	1360	RT, MS
153	Monoterpene ethyl ether	tr	tr	1368	
154	*α*-Copaene	tr	tr	1378	RT, MS
155	Nonanal diethyl acetal	tr	tr	1382	RT, MS
156	Ethyl decanoate	tr	tr	1382	RT, MS
157	*β*-Elemene	tr	tr	1388	RT, MS
158	Limonen-10-yl acetate	tr	tr	1388	RT, MS
159	Dodecanal	tr	tr	1389	RT, MS
160	1-Tetradecene	tr	tr	1390	RT, MS
161	Decyl acetate	tr	tr	1393	RT, MS
162	p-Menth-1-en-9-yl acetate	tr	tr	1398	RT, MS
163	Citronellal diethyl acetal	tr	tr	1398	RT, MS
164	Tetradecane	tr	tr	1400	RT, MS
165	(*Z*)-*α*-Bergamotene	tr	tr	1419	RT, MS
166	*β*-Caryophyllene	0.26	0.27	1422	RT, MS
167	*α*-Santalene	tr	tr	1423	RT, MS
168	Geranyl acetone	tr	tr	1430	RT, MS

Continued on next page.

Downloaded by STANFORD UNIV GREEN LIBR on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch013

Table II. (Continued). Volatile Composition of Fresh and Aged Limoncello[a]

	Component	*Fresh FID%*	*Aged FID%*	*Kovats*	*Confirmation[b]*
169	(*E*)-*α*-Bergamotene	tr	tr	1435	RT, MS
170	Aromadendrene	tr	tr	1443	RT, MS
171	epi-*β*-Santalene	tr	tr	1449	RT, MS
172	*γ*-Muurolene	tr	tr	1450	RT, MS
173	(*E*)-*β*-Farnesene	tr	tr	1453	RT, MS
174	*α*-Humulene	tr	tr	1455	RT, MS
175	Geranyl propionate	tr	tr	1468	RT, MS
176	*β*-Santalene	tr	tr	1472	RT, MS
177	*α*-Selinene	tr	tr	1477	RT, MS
178	Decanal diethyl acetal	tr	tr	1482	RT, MS
179	Selina-3,11-diene	tr	tr	1488	RT, MS
180	Valencene	0.15	0.13	1492	RT, MS
181	Bicyclogermacrene	0.17	0.04	1494	RT, MS
182	(*Z*)-*α*-Bisabolene	tr	tr	1496	RT, MS
183	(*E*,*E*)-*α*-Farnesene	tr	tr	1497	RT, MS
184	Tridecanal	tr	tr	1498	RT, MS
185	*β*-Bisabolene	0.49	0.76	1504	RT, MS
186	5,9-Dimethyl-2,4,8-decatrienoic acid	tr	tr	1506	RT, MS
187	*γ*-Bisabolene i	tr	tr	1510	RT, MS
188	*γ*-Bisabolene ii	tr	tr	1512	RT, MS
189	Monoterpene ethyl ether	tr	tr	1515	
190	Monoterpene ethyl ether	tr	tr	1518	
191	*σ*-Cadinene	tr	tr	1519	RT, MS
192	Sesquisabinene hydrate	tr	tr	1529	RT, MS
193	(*E*)-*α*-Bisabolene	tr	tr	1541	RT, MS
194	Dodecanoic acid	tr	tr	1545	RT, MS
195	(*E*)-Nerolidol	tr	tr	1549	RT, MS
196	Undecanal diethyl acetal	tr	tr	1576	RT, MS
197	Ethyl dodecanoate	tr	tr	1578	RT, MS
198	Sesquiterpene oxygenated compound	tr	tr	1595	
199	Hexadecane	tr	tr	1600	RT, MS
200	t-Cadinol	tr	tr	1630	RT, MS
201	*α*-Cadinol	tr	tr	1635	RT, MS
202	2-Hydroxy-*β*-santalene	tr	tr	1640	RT, MS
203	Campherenol	tr	tr	1655	RT, MS
204	*α*-Bisabolol	tr	tr	1671	RT, MS
205	Pentadecanal	tr	tr	1695	RT, MS
206	(*E*,*E*)-Farnesal	tr	tr	1719	RT, MS
207	Tetradecanoic acid	tr	tr	1738	RT, MS
208	Nootkatone	tr	tr	1767	RT, MS
209	Ethyl tetradecanoate	tr	tr	1777	RT, MS
210	Hexadecanal	tr	tr	1823	RT, MS

Continued on next page.

Downloaded by STANFORD UNIV GREEN LIBR on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch013

Table II. (Continued). Volatile Composition of Fresh and Aged Limoncello[a]

	Component	*Fresh FID%*	*Aged FID%*	*Kovats*	*Confirmation*[b]
211	Pentadecanoic acid	tr	tr	1845	RT, MS
212	Ethyl pentadecanoate	tr	tr	1877	RT, MS
213	Heptadecanal	tr	tr	1895	RT, MS
214	Limettin	tr	tr	1905	RT, MS
215	Hexadecanoic acid	tr	tr	1943	RT, MS
216	Ethyl hexadecanoate	tr	tr	1977	RT, MS
217	Ethyl heptadecanoate	tr	tr	2075	RT, MS
218	7-isoPropenylcoumarin	tr	tr	2110	MS
219	Linoleic acid	tr	tr	2125	RT, MS
220	Linolenic acid	tr	tr	2132	RT, MS
221	Oleic acid	tr	tr	2133	RT, MS
222	Ethyl linoleate	tr	tr	2146	RT, MS
223	Ethyl linolenate	tr	tr	2151	RT, MS
224	Ethyl oleate	tr	tr	2176	RT, MS
225	Ethyl octadecanoate	tr	tr	2177	RT, MS
226	Docosane	tr	tr	2200	RT, MS
227	Tricosane	tr	tr	2300	RT, MS
228	Squalene	tr	tr	2719	RT, MS

[a] Concentrations given were determined by GC-FID adjusted using an internal standard and applied correction factors. [b] Kovats = Standard retention data using an alkane homologous series on non-polar column. [c] tr = trace component. [d] RT = Retention time on 50m OV1 column. [e] MS = Mass spectrum (EI, 70eV). [f] nd = not detected.

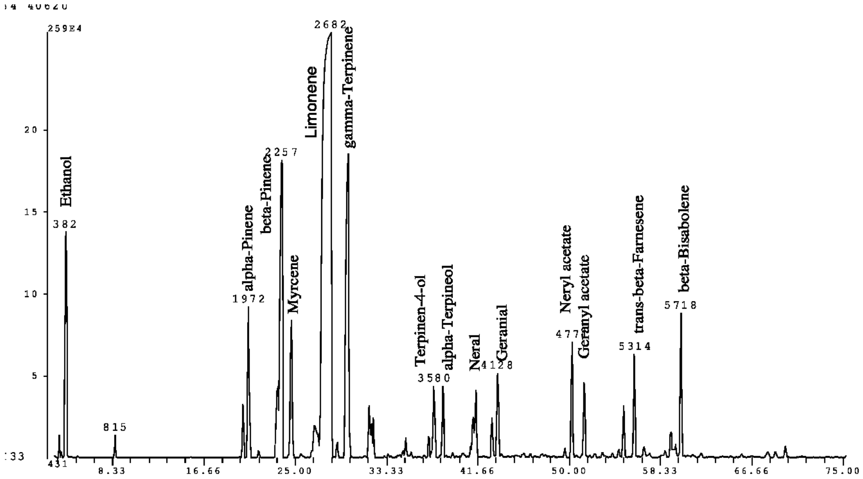


Figure 1. Aged Limoncello liquid/liquid extract profile.

Downloaded by STANFORD UNIV GREEN LIBR on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch013

Table III. Key Volatile Components of Limoncello by GC-O

	Component	*Fresh FID%*	*Aged FID%*	*Descriptor*
1	Acetaldehyde	tr	tr	ethereal, pungent
13	Valeraldehyde	tr	tr	weak cream
15	Acetaldehyde diethyl acetal	0.02	0.05	fresh, ethereal
19	Butyric acid	tr	tr	rancid butter
26	isoValeric acid	tr	tr	acid, cheese, sweaty
27	(*Z*)-3-Hexenol	tr	tr	green grass
34	*α*-Thujene	0.44	0.26	terpene like
35	*α*-Pinene	1.92	1.09	terpene, coniferous
36	Benzaldehye	tr	tr	weak almond
39	Camphene	0.08	0.04	camphoraceous
42	Hexanoic acid	tr	tr	weak acid, fatty
44	Sabinene	2.29	0.74	woody, spicy, oily
45	*β*-Pinene	19.13	6.68	terpene like, coniferous
46	Octanal	0.03	0.01	aldehydic, peely, green
47	*β*-Myrcene	1.20	1.71	terpene like, citrus, soapy
50	*α*-Phellandrene	0.02	0.03	terpene, citrusy
54	*α*-Terpinene	0.05	0.04	terpene like, slightly citrus
55	p-Cymene	0.36	0.83	harsh, gasoline, terpene
58	Limonene	54.05	52.47	orange peel
59	(*Z*)-*β*-Ocimene	0.70	0.60	terpene, slightly floral
60	Acetophenone	tr	tr	weak aromatic, almond
61	Melonal	tr	tr	weak cucumber, green
62	(*E*)-*β*-Ocimene	0.13	0.08	terpene, slightly floral
63	*γ*-Terpinene	12.22	8.09	citrus, lime, green, soapy
64	(*E*)-Sabinene hydrate	0.20	0.10	citrus like
67	p-*α*-Dimethylstyrene	tr	tr	pine, spicy, plastic
68	Terpinolene	0.24	0.27	terpene, slightly citrus
72	Linalool	0.32	0.19	floral, citrus
76	Fenchol	tr	tr	weak camphoraceous
78	(*Z*)-p-Mentha-2,8-dienol	0.03	0.02	terpene, citrus
82	Camphor	0.02	0.02	camphoraceous
86	(*E*)-p-Menth-2-enol	0.03	0.03	terpene, citrus
87	(*E*)-Sabinene hydrate et ether	nd	0.08	herbal citrus
89	Citronellal	0.09	0.03	citrus, green, soapy
95	Borneol	0.01	0.01	camphoraceous
97	(*Z*)-Sabinene hydrate et ether	nd	0.09	herbal, citrus
98	Octanoic acid	tr	tr	weak buttery, cheesy
99	Limonen-4-ol	0.03	0.37	citrus
100	p-Cymen-8-ol	0.02	tr	sharp herbal, cumin, oily
101	Terpinen-4-ol	0.14	0.02	sweet, green, musty
105	*alpha;*-Terpineol	0.46	0.36	floral, earthy
106	p-Menth-2-enyl ethyl ethernd	nd	0.01	herbal, citrus

Continued on next page.

Table III. (Continued). Key Volatile Components of Limoncello by GC-O

	Component	*Fresh FID%*	*Aged FID%*	*Descriptor*
110	Decanal	0.04	0.02	waxy, floral, orange like
111	Benzothiazole	tr	tr	weak vegetative, rubbery
119	Terpinen-4-yl ethyl ether	tr	tr	herbal, citrus, berry like
120	Nerol	tr	tr	floral, rose
122	Neral	0.79	0.49	fresh, citrus
124	Geraniol	0.19	0.31	floral, rose
126	Geranial	1.05	0.57	fresh, lemon
128	α-Terpinyl ethyl ether	0.01	0.04	coniferous, slightly citrus
132	Bornyl acetate	0.03	0.02	camphoraceous, woody
135	Carvacrol	0.05	0.01	spicy, phenolic
145	α-Terpinyl acetate	tr	tr	woody, citrus, spicy
146	β-Citronellyl acetate	tr	tr	citrus, passionfruit
147	Neryl acetate	0.39	0.54	floral, rose
151	Geranyl acetate	0.50	0.30	green, fruity, floral
165	β-Caryophyllene	0.26	0.27	spicy, woody, oily
179	Valencene	0.15	0.13	grapefruit, citrus, oily
180	Bicyclogermacrene	0.17	0.04	woody, spicy
184	β-Bisabolene	0.49	0.76	woody, spicy

Conclusions

Compositionally, the major differences indicated by the analyses appeared to be the reduction in concentration of β-pinene and γ-terpinene as they hydrolyzed/oxidized in the aged sample. β-Pinene may have formed compounds like pinan-2-ol and γ-terpinene. In turn γ-terpinene hydrolyzes to terpinen-4-ol. In excess ethanol, the latter formed a low concentration ofthe corresponding ethyl ether (Figure 2).

Other terpene alcohols susceptible to ethyl ether formation were α-terpineol, p-menth-2-enol and sabinene hydrate (Figure 3).

The presence of aldehydes in excess ethanol led to the formation of diethyl acetals such as citronellal diethyl acetal and several aliphatic aldehyde diethyl acetals. In addition some ester formation occurred as ethanol reacted with low concentration acids.

Citral (neral and geranial) is widely known to be unstable in citrus oils and juice on exposure to air, light, heat and acid (*15–20*). It is reported to rearrange and reduce to components such as p-cymene, p-cymen-8-ol, p-methyl-acetophenone and p-α-dimethylstyrene (Figure 5). No p-methylacetophenone was detected in either extract. In the agedLimoncello the neral and geranial concentrations had reduced after five months but they had not disappeared, as observed in aged juices or acidic carbonated beverages.

Organoleptically, the fresh Limoncello concentrated extract was described as strongly citral, fresh, lemon curd. The aged concentrate extract was more oxidized

Downloaded by STANFORD UNIV GREEN LIBR on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch013

Downloaded by STANFORD UNIV GREEN LIBR on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch013

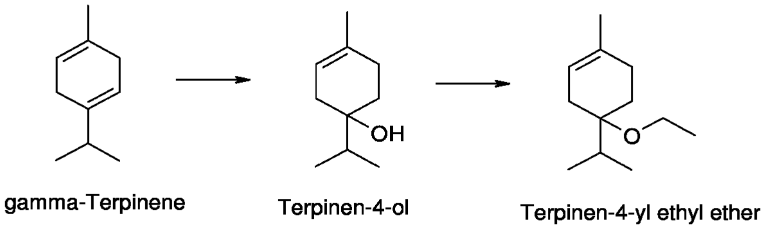


Figure 2. Ethyl ether formation from gamma-terpinene.

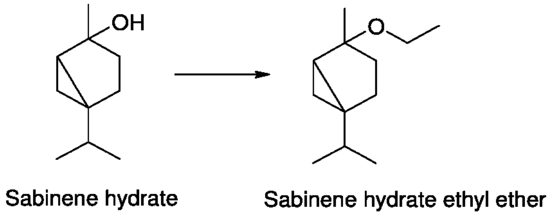


Figure 3. Ethyl ether formation from sabinene hydrate.

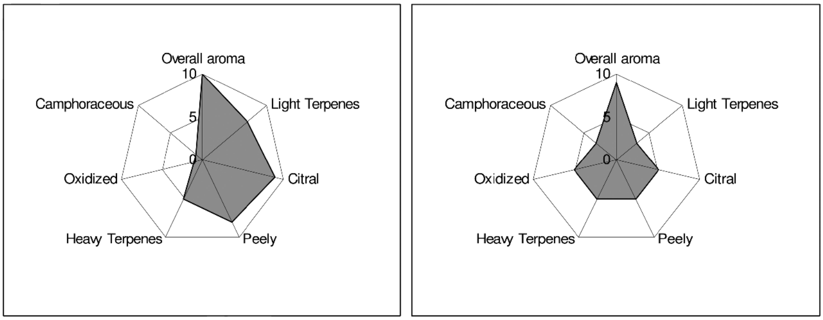


Figure 4. Fresh and Aged Limoncello Sensory Spider graphs.

lemon, less fresh, heavy lemon, missing lower volatiles, lower citral.This aroma evaluation and the GC-Olfactometry work corresponded with the analyses. The biggest difference detected was a reduction in the concentration of the highly volatile monoterpenes as they became oxidized, which gave rise to the loss of fresh citrusy notes. In addition the harsh gasoline, oxidized terpene note of p-cymene was increased, which had a negative impact on the aged sample (Figure 4).

The various ethyl ethers formed were not perceived as giving significantlynegative notes to the aged sample. The presence of citral after five months aided the Limoncello, in still giving a positively perceived aroma. It is possible that the emulsion in ethanol helped to stabilize the citral to some extent due to hydrophobic shielding, thus increasing shelf life.

Thus, for the connoisseur Limoncello is best stored refrigerated to prolong its fresh aroma and shelf life. However its storage at room temperature for many months does not appear to produce as many significant off-notes compared to more

Downloaded by STANFORD UNIV GREEN LIBR on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch013

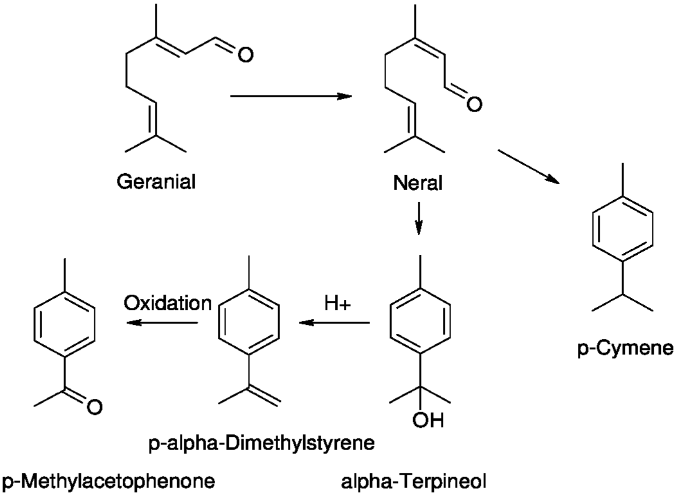


Figure 5. Rearrangement products of citral.

acidic fruit juices or carbonated beverages. Liquor shops still store and sell them at ambient temperature in clear glass bottles.

References

1. Nota, G.; Navaglio, D.; Romano, R.; Sabia, V.; Attanasio, G.; Spagna, M. S. Control of the lemon peel maceration step in the preparation of lemon liquor. *Ind. Bevande* **1999**, *28*, 159, 1–5.
2. Versari, A.; Natali, N.; Russo, M. T.; Antonelli, A. Analysis of some Italian lemon liquors (Limoncello). *J. Agric. Food Chem.* **2003**, *51*, 4978–4983.
3. Crupi, M. L.; Costa, R.; Dugo, P.; Dugo, G.; Mondello, L. A comprehensive study on the chemical and aromatic characteristics of lemon liquor. *Food Chem.* **2007**, *105*, 771–783.
4. Schieberle, P.; Grosch, W. J. Identification of potent flavor compounds formed in an aqueous lemon oil/citric acid emulsion. *J. Agric. Food Chem.* **1988**, *36*, 797–800.
5. Bononi, M.; Lubian, E.; Puleo, L.; Tateo, F.; Fasan, S. Criterion of PCA characterization of Limoncello liquors. *Ind. Bevande* **2001**, *30*, 174, 371–374.
6. Naviglio, D.; Ferrara, L.; Montesano, D.; Mele, G.; Naviglio, B.; Tomaselli, M. New solid−liquid extraction technology for the production of lemon liquor. *Ind. Bevande* **2001**, *30*, 362–370.
7. Naviglio, D.; Pizzolongo, F.; Mazza, A.; Montuori, P.; Triassi, M. Chemical−physical study of alcoholic extract of Flavedo lemon and essential lemon oil to determine microbic change responsible for Limoncello turbidity. *Ind. Bevande* **2005**, *34*, 425–431.

8. Naviglio, D.; Raia, C.; Bergna, M.; Saggiomo, S. Stability control and genuineness evaluation of lemon liquor: Use of dynamic headspace. *Ind. Bevande* **2003**, *32*, 184, 121–126.
9. Moio, L.; Piombino, P.; Di Marzio, L.; Incoronato, C.; Addeo, F. Lemon liqueur aroma. *Ind. Bevande* **2000**, *29*, 169, 499–506.
10. Dugo, P.; Russo, M.; Mondello, L.; Dugo, G.; Postorino, S.; Carnacini, A. Chemical and physico−chemical parameters and composition of the aromatic fraction of Limoncello. *Ital. J. Food Sci.* **2000**, *12*, 343–351.
11. Grisco, J. Limoncello Recipe. *The Washington Post*, December 7, 2009.
12. Poiana, M.; Attanasio, G.; Albanese, D.; Di Matteo, M. Alcoholic extracts composition from lemon fruits of the Amalfi-Sorrento peninsula. *J. Ess. Oil Res.* **2006**, *18*, 432–437.
13. Ayedoun, A. M.; Sossou, P. V.; Mardarowicz, M.; Leclercq, P. A. Volatiles constituents of the peel and leaf oils of citrus limon L. Burm. F. from Benin. *J. Ess. Oil Res.* **1996**, *8*, 441–444.
14. Boelens, M. H.; Jimenez, R. The chemical composition of some Mediterranean citrus oils. *J. Ess. Oil Res.* **1989**, *1*, 151–159.
15. Ueno, T.; Matsuda, T.; Ho, C. T. Formation mechanism of *p*-methylacetophenone from citral via a *tert*-alkoxy radical intermediate. *J. Agric. Food Chem.* **2004**, *52*, 5677–5684.
16. Djordjevic, D.; Ceracaci, L.; Alamed, J.; McClements, D. J.; Decker, E. A. Chemical and physical stability of citral and limonene in sodium dodecyl sulfate-chitosan and gum Arabic-stabilized oil-in-water emulsions. *J. Agric. Food Chem.* **2007**, *55*, 3585–3591.
17. Liang, C. P.; Mingfu, W.; Simon, J. E.; Ho, C. T. Antioxidant activity of plant extracts on the inhibition of citral off-odor formation. *Mol. Nutr. Food Res.* **2004**, *48*, 308–317.
18. Nguyen, H.; Campi, E. M.; Jackson, W. R.; Patti, A. F. Effect of oxidative deterioration on flavour and aroma components of lemon oil. *J. Food Chem.* **2009**, *112*, 2, 388–393.
19. Barton, D. H. R.; Parekh, S. I. A short and efficient method for the preparation of α, *p*-dimethylstyrene from citral. *Syn. Comm.* **1989**, *19*, 3353–3361.
20. Neumann, M.; Garcia, N. A. Kinetics and mechanism of the light-induced deterioration of lemon oil. *J. Agric. Food Chem.* **1992**, *40*, 957–960.

Downloaded by STANFORD UNIV GREEN LIBR on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ch013

Subject Index

A

Acetaldehyde, in volatile composition analysis of limoncello, 183*t*, 189*t*
Acetaldehyde butan-2,3-diol acetal, in volatile composition analysis of limoncello, 183*t*
Acetaldehyde diethyl acetal, in volatile composition analysis of limoncello, 183*t*, 189*t*
Acetic acid
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 183*t*
Acetone, in volatile composition analysis of limoncello, 183*t*
Acetophenone, in volatile composition analysis of limoncello, 183*t*, 189*t*
Acyl carrier protein, 117
Air-water partition coefficient, effect on H_2O-ethanol solution system odor release, 166
Alcoholic beverages, 17
  polyphenols in, 17
  red wine, 17
    resveratrol structure in, 17, 17*f*
Alcoholic beverages odorant release, 161
α-Bisabolol
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 183*t*
α-Cadinol, in volatile composition analysis of limoncello, 183*t*
α-Copaene, in volatile composition analysis of limoncello, 183*t*
α-Cubebene, in volatile composition analysis of limoncello, 183*t*
α-Fenchene, in volatile composition analysis of limoncello, 183*t*
α-Humulene
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 183*t*
α,p-Dimethylstyrene, reaction from citral to potent off notes, 145*f*
α-Phellandrene
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 183*t*, 189*t*
α-Pinene
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 183*t*, 189*t*
α-Santalene, in volatile composition analysis of limoncello, 183*t*
α-Selinene, in volatile composition analysis of limoncello, 183*t*
α-Terpinene
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 183*t*, 189*t*
α-Terpineol
  formation in lemon emulsion with citral formulation, 154, 157*f*
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  reaction from citral to potent off notes, 145*f*
  in volatile composition analysis of limoncello, 183*t*, 189*t*
α-Terpinyl acetate, in volatile composition analysis of limoncello, 183*t*, 189*t*
α-Terpinyl ethyl ether, in volatile composition analysis of limoncello, 183*t*, 189*t*

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ix002

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ix002

α-Thujene
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 183*t*, 189*t*
Amino acid
  related analogues in tea, 35*f*
  taste active compounds in tea, 36*t*, 37
Anthocyanin content
  of blackberry juice, 138*f*, 139
  of blueberry juice, 135, 137, 138*f*, 139
  of muscadine grapes, 138*f*, 139
  of strawberry juice, 138*f*, 139
Anti-cancer, as bio-function properties of polyphenols in black tea, 54
Anti-inflammation, as bio-function properties of polyphenols in black tea, 51
Apple juice, 13
Apple juice aroma
  components
    butyl acetate, 105, 106*t*, 107, 109*t*, 111*t*, 112*t*
    (E)-2-hexenal, 105, 106*t*, 107, 109*t*, 111*t*, 112*t*
    (E)-2-hexenol, 105, 106*t*, 107, 109*t*, 111*t*, 112*t*
    ethyl butyrate, 105, 106*t*, 107, 109*t*, 111*t*, 112*t*
    ethyl 2-methylbutyrate, 105, 106*t*, 107, 109*t*, 111*t*, 112*t*
    hexanal, 105, 106*t*, 107, 109*t*, 111*t*, 112*t*
    hexanol, 105, 106*t*, 107, 109*t*, 111*t*, 112*t*
    hexyl acetate, 105, 106*t*, 107, 109*t*, 111*t*, 112*t*
    2-methylbutanol, 105, 106*t*, 107, 109*t*, 111*t*, 112*t*
    2-methylbutyl acetate, 105, 106*t*, 107, 109*t*, 111*t*, 112*t*
  evaluation of, 103
  odor threshold values in water, 105, 106*t*
    β-damascenone, 106
  profiles analyzed and evaluated, 110, 112*t*, 113*t*
Argentinian lemon single fold oil chromatogram, 10, 12*f*
Aroma extract dilution analysis, results for Sri Lankan black teas, 28
Aroma index model
  application of, 108
    commercial apple juices from concentrate, 110, 111, 112*f*
    NFC juices, 108
  derived standardization factors, 107, 111*t*
  description of aroma restoration, 103
  development of, 105, 107, 109*t*
Aromadendrene, in volatile composition analysis of limoncello, 183*t*
Ascardole epoxide, in volatile composition analysis of limoncello, 183*t*
Ascaridole, in volatile composition analysis of limoncello, 183*t*
Ascorbic acid, for maximum stability of folate, 119
Aspalathin, 71*f*, 72, 72*t*
Aspalathus linearis extract
  sensory evaluation using LC Taste®, 71*f*, 72
    fractionation results and contained compounds, 72, 72*t*
  taste modulating compounds aspalathin and nothofagin, 71*f*, 72
Astringent, taste thresholds data in teas, 38*t*, 39

B

Benzaldehye, in volatile composition analysis of limoncello, 183*t*, 189*t*
Benzoic acid, in lemon peel ethanolic extract volatile component analysis, 179*t*
Benzothiazole, in volatile composition analysis of limoncello, 183*t*, 189*t*
Berry flavors

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ix002

- strawberry sweet charley liquid/liquid extract, 12, 14*f*
- volatile/non-volatile components of, 12

β-Alanine, 117
β-Bisabolene
- in lemon peel ethanolic extract volatile component analysis, 179*t*
- in volatile composition analysis of limoncello, 183*t*, 189*t*

β-Caryophyllene
- in lemon peel ethanolic extract volatile component analysis, 179*t*
- in volatile composition analysis of limoncello, 183*t*, 189*t*

β-Citronellyl acetate
- in lemon peel ethanolic extract volatile component analysis, 179*t*
- in volatile composition analysis of limoncello, 183*t*, 189*t*

β-Cyclodextrin, 146
- effect of protective and pseudophase demonstration with citral, 155*f*
- lemon emulsion system in sensory evaluation with, 156*f*
- SPME from emulsion with, 157*f*

β-Damascenone, odor threshold values in water of apple juice aroma, 106
β-Damascenone odorant, 98*t*, 99
β-Elemene, in volatile composition analysis of limoncello, 183*t*
β-Myrcene
- in lemon peel ethanolic extract volatile component analysis, 179*t*
- in volatile composition analysis of limoncello, 183*t*, 189*t*

β-Phellandrene, in volatile composition analysis of limoncello, 183*t*
β-Pinene
- in lemon peel ethanolic extract volatile component analysis, 179*t*
- in volatile composition analysis of limoncello, 189*t*

β-Santalene, in volatile composition analysis of limoncello, 183*t*
Beverages
- alcoholic, 17
- coffee, 8
- dairy, 15
- flavored water, 15
- fruit juices/oils, 9, 12
- global consumption by category from 2003 to 2008, 5*t*
- global growth by region from 2003 to 2008, 5*t*
- non-carbonated, overview of flavors in, 3
- North American consumption by category from 2003 to 2008, 6*t*
- protected and unprotected sensory evaluation, 152, 153*f*, 155*f*
- sports/energy drinks, 15
- tea, 4

Bicyclogermacrene
- in lemon peel ethanolic extract volatile component analysis, 179*t*
- in volatile composition analysis of limoncello, 183*t*, 189*t*

Bitter, taste thresholds data in teas, 38*t*, 39
Black tea
- bio-function properties of polyphenols in, 50
  - anti-cancer, 54
  - anti-inflammation, 51
  - taste, 50
- contribution of polyphenols in, 45, 46

Blackberries, processing effects on, 139
- anthocyanin content, 138*f*, 139

Blueberry juice
- anthocyanin content, 135, 137, 138*f*, 139
- consumer acceptability/evaluation test, 135, 137*t*
- HHP treatment effects on quality of, 137, 138*f*
- physiochemical characteristics of, 135, 136*t*

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ix002

  processing effects on flavor and quality of, 129, 139
  thermal processing effects on
    bioactivity of, 134
    color and flavor of, 135
  total ORAC as function of processing methods, 134, 136*f*
  total phenolic content as function of processing methods, 134, 135*f*
  See also Fruit juices
Borneol, in volatile composition analysis of limoncello, 183*t*, 189*t*
Bornyl acetate, in volatile composition analysis of limoncello, 183*t*, 189*t*
Bovine serum albumin surface tension, 172, 172*t*
Bulk phase mass transfer mechanism, 167, 168, 169
Butan-2-one, in volatile composition analysis of limoncello, 183*t*
3-Buten-2-one, in volatile composition analysis of limoncello, 183*t*
Butyl acetate, apple juice aroma component, 105, 106*t*, 109*t*, 111*t*, 112*t*
Butyric acid, in volatile composition analysis of limoncello, 183*t*, 189*t*

C

Caffeine
  in coffee, 8
  in sports/energy drinks, 15
  in tea, 5, 7*f*
Californian grapefruit five fold oil chromatogram, 11, 13*f*
Camphene
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 183*t*, 189*t*
Campherenol, in volatile composition analysis of limoncello, 183*t*
Camphor
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 183*t*, 189*t*
Carvacrol, in volatile composition analysis of limoncello, 183*t*, 189*t*
Catechins
  astringency and bitterness in black tea, 50, 52*t*
  (-)-epicatechin, 46
  (-)-epicatechin gallate, 46
  (-)-epigallocatechin, 46
  (-)-epigallocatechin-3-gallate, 46
  See also Polyphenols
Cations, taste active compounds in tea, 36*t*, 37
Cherry flavors, 13
Chlorogenic acid, in tea, 7, 8*f*
  and its analogues, 8*f*
1,8-Cineole, in volatile composition analysis of limoncello, 183*t*
cis-Bovolide. *See* 3,4-Dimethyl-cis-5-pentylidene-2,5-dihydrofuran-2-one
Citral
  beverage system, 148*t*, 150
  mechanism of off-note formation, 145*f*
    α,p-dimethylstyrene, 145*f*
    α-terpineol, 145*f*
    disproportionnation, 145*f*
    geranial, 145*f*
    8-hydroperoxy-p-cymene, 145*f*
    neral, 145*f*
    oxidation, 145*f*
    p-cresol, 145*f*
    p-cymene, 145*f*
    p-cymen-8-ol, 145*f*
    p-menthadien-8-ols, 145*f*
    p-methylacetophenone, 145*f*
  protective and pseudophase demonstration of β-cyclodextrin effect with, 155*f*
  stability by cyclodextrin micro emulsion technology in juice beverages, 143
Citronellal

- in lemon peel ethanolic extract volatile component analysis, 179*t*
- in volatile composition analysis of limoncello, 183*t*, 189*t*

Citronellal diethyl acetal, in volatile composition analysis of limoncello, 183*t*

Coffea arabica, 8
- stir-bar sorption extraction chromatograms, 8, 9*f*

Coffea canephora, 8
- Medium Roast Kenyan and Medium Roast Indo Robusta beans comparison, 83, 86*f*
- negatively perceived contributors in, 81, 84*f*
- stir-bar sorption extraction chromatograms, 8, 10*f*

Coffee, 8
- *Coffea arabica*, 8
  - stir-bar sorption extraction chromatograms, 8, 9*f*
- *Coffea canephora*, 8
  - stir-bar sorption extraction chromatograms, 8, 10*f*
- key odorants of, 77, 82*t*
- RTD, global consumption by category from 2003 to 2008, 8, 9*t*
- spider charts profile of, 84*f*
- sugar degradation products, 83, 87*f*
- volatile/non-volatile components of, 8, 9*f*, 10*f*

Cold press fruit oils, 10
- Persian lime cold press oil chromatogram, 11, 12*f*

p-Cresol
- organoleptic property of odor thresholds in water, 146*t*
- reaction from citral to potent off notes, 145*f*

Critical micelle concentration, 146

Cyclodextrins, 144
- geometry and physical dimension models of
  - hydrophilic exterior, 144, 146*f*
  - hydrophobic core, 144, 146*f*
- guest equilibrium in solution, 146, 147*f*
- micro emulsion technology
  - in juice beverages of citral stability, 143

Cyclopia intermedia extract
- sensory evaluation and coupled
  - taste dilution analysis using LC Taste®, 68
  - fractionation results and contained compounds, 68, 69*t*
  - LC Taste® chromatogram of, 68, 68*f*
- taste modulating compounds
  - identification using LC Taste®, 69
  - hesperidin and mangiferin, 69, 69*t*, 70*f*
- TMP values for, 69, 70*f*

Cyclotene
- component in coffee, 82*t*
- compound formed during sugar degradation process, 83, 87*f*

p-Cymene
- organoleptic property of odor thresholds in water, 146*t*
- reaction from citral to potent off notes, 145*f*

p-Cymen-8-ol
- organoleptic property of odor thresholds in water, 146*t*
- for SPME results, 149*f*

D

Dairy beverages, 15

Decanal
- in lemon peel ethanolic extract volatile component analysis, 179*t*
- in volatile composition analysis of limoncello, 183*t*, 189*t*

Decanal diethyl acetal, in volatile composition analysis of limoncello, 183*t*

Decane, in volatile composition analysis of limoncello, 183*t*

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ix002

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ix002

Decyl acetate, in volatile composition analysis of limoncello, 183*t*
δ-Cadinene, in volatile composition analysis of limoncello, 183*t*
δ-Car-3-ene, in volatile composition analysis of limoncello, 183*t*
δ-Terpineol, in volatile composition analysis of limoncello, 183*t*
Diethyl oxalate, in volatile composition analysis of limoncello, 183*t*
Difurfuryl sulfide, component in coffee, 82*t*
Dimbula Sri Lankan black teas
  AEDA results, 28, 29
  characteristic odorants of, 21
  chiral compound enantiomers, ratios of, 28*t*, 29
  FD factors of aroma compounds in, 27*t*, 28
  functional groups in
    spider charts, 26, 26*f*
    stacked bar graph, 24*f*, 26
  3HDMF concentrations in, 29*f*, 30
  4HDMF in
    calibration curves of, 23*t*
    concentrations of, 29*f*, 30
  sensory characteristics of, 25
4,7-Dime-bicyclo[3.2.1]oct-3-en-6-one, in volatile composition analysis of limoncello, 183*t*
2.6-Dimethoxy-4-vinylphenol, in lemon peel ethanolic extract volatile component analysis, 179*t*
Dimethyl disulfide, component in coffee, 82*t*
Dimethyl trisulfide, component in coffee, 82*t*
3,4-Dimethyl-cis-5-pentylidene-2,5-dihydrofuran-2-one, compound of Kenyan brew coffee, 84, 88*f*
5,9-Dimethyl-2,4,8-decatrienoic acid, in volatile composition analysis of limoncello, 183*t*
2,5-Dimethyl-4-hydroxy-3(2H)-furanone, component of Medium Roast Kenyan and Medium Roast Indo Robusta beans, 83, 86*t*
4,5-Dimethyl-3-hydroxy-2(5h)-furanone, component of Medium Roast Kenyan and Medium Roast Indo Robusta beans, 83, 86*t*
Disproportionnation, reaction from citral to potent off notes, 145*f*
Docosane, in volatile composition analysis of limoncello, 183*t*
Dodecanal, in volatile composition analysis of limoncello, 183*t*
Dodecane, in volatile composition analysis of limoncello, 183*t*
Dodecanoic acid, in volatile composition analysis of limoncello, 183*t*

E

(E)-α-Bergamotene
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 183*t*
(E)-α-Bisabolene, in volatile composition analysis of limoncello, 183*t*
Earthy aroma, 96, 97*f*, 97*t*
(E)-β-Ocimene, in lemon peel ethanolic extract volatile component analysis, 179*t*
(E)-β-Damascenone, component in coffee, 82*t*
(E)-β-Farnesene
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 183*t*
(E)-β-Ocimene, in volatile composition analysis of limoncello, 183*t*, 189*t*
(E)-Carveol, in volatile composition analysis of limoncello, 183*t*
(E)-Carvyl acetate, in volatile composition analysis of limoncello, 183*t*

(E)-4,8-Dimethyl-1,3,7-nonatriene, in volatile composition analysis of limoncello, 183*t*
(E,E)-α-Farnesene, in volatile composition analysis of limoncello, 183*t*
(E,E)-Farnesal, in volatile composition analysis of limoncello, 183*t*
(E)-2-Hexenal, apple juice aroma component, 105, 106*t*, 109*t*, 111*t*, 112*t*
(E)-2-Hexenol
  apple juice aroma component, 105, 106*t*, 109*t*, 111*t*, 112*t*
  in volatile composition analysis of limoncello, 183*t*
Electrospray ionization, 119
Emulsion systems, 154
Enantiomers ratio, of chiral compounds in Sri Lankan black teas
  γ-decalactone enantiomers ratio, 28*t*, 29
  γ-nonalactone enantiomers ratio, 28*t*, 29
(E)-Nerolidol, in volatile composition analysis of limoncello, 183*t*
(E)-2-Nonenal, component in coffee, 82*t*
Enzymatic release, for fruit juice production, 131
epi-β-Santalene, in volatile composition analysis of limoncello, 183*t*
(-)-Epicatechin, polyphenol in green tea, 46, 48*f*
(-)-Epicatechin gallate, polyphenol in green tea, 46, 48*f*
(-)-Epigallocatechin, polyphenol in green tea, 46, 48*f*
(-)-Epigallocatechin-3-gallate, polyphenol in green tea, 46, 48*f*
(E)-p-Mentha-1(7),8-dien-2-ol, in volatile composition analysis of limoncello, 183*t*
(E)-p-Mentha-2,8-dienol, in volatile composition analysis of limoncello, 183*t*
(E)-p-Menth-2-enol, in volatile composition analysis of limoncello, 183*t*, 189*t*
2,3-Epoxygeranial i, in volatile composition analysis of limoncello, 183*t*
2,3-Epoxygeranial ii, in volatile composition analysis of limoncello, 183*t*
Eriodictyol, 63*f*, 65*t*
Eriodicyton angustifolium extract
  sensory evaluation and coupled
    taste dilution analysis using LC Taste®, 67
    fractionation results and
      contained compounds, 65*t*, 67
    LC Taste® chromatogram of,
      66*f*
  taste modulating compounds
    homoeriodictyol, sterubin,
      hesperetin, eriodictyol, 63*f*, 65*t*, 67
  TMP values for, 66*f*, 67
(E)-Sabinene hydrate
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 183*t*, 189*t*
(E)-Sabinene hydrate et ether, in volatile composition analysis of limoncello, 189*t*
(E)-Sabinene hydrate ethyl ether, in volatile composition analysis of limoncello, 183*t*
(E)-Terpin hydrate, in volatile composition analysis of limoncello, 183*t*
Ethanol surface tension, 172, 172*t*
Ethyl acetate, in volatile composition analysis of limoncello, 183*t*
Ethyl 2-butenoate, 168*t*, 170, 170*t*, 171*f*, 171*t*
Ethyl butyrate, 168*t*, 169*f*
  apple juice aroma component, 105, 106*t*, 109*t*, 111*t*, 112*t*
Ethyl cyclotene, compound formed during sugar degradation process, 83, 87*f*

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ix002

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ix002

Ethyl decanoate, in volatile composition analysis of limoncello, 183*t*
Ethyl dodecanoate, in volatile composition analysis of limoncello, 183*t*
Ethyl heptadecanoate, in volatile composition analysis of limoncello, 183*t*
Ethyl hexadecanoate, in volatile composition analysis of limoncello, 183*t*
Ethyl hydrogen succinate, in volatile composition analysis of limoncello, 183*t*
3-Ethyl 2-hydroxy-2-cyclopenten-1-one, component of Medium Roast Kenyan and Medium Roast Indo Robusta beans, 83, 86*t*
Ethyl linoleate, in volatile composition analysis of limoncello, 183*t*
Ethyl linolenate, in volatile composition analysis of limoncello, 183*t*
Ethyl 3-methyl-1-butenyl ether, in volatile composition analysis of limoncello, 183*t*
Ethyl 2-methylbutyrate, apple juice aroma component, 105, 106*t*, 109*t*, 111*t*, 112*t*
Ethyl octadecanoate, in volatile composition analysis of limoncello, 183*t*
Ethyl oleate, in volatile composition analysis of limoncello, 183*t*
Ethyl pentadecanoate, in volatile composition analysis of limoncello, 183*t*
Ethyl pyruvate, in volatile composition analysis of limoncello, 183*t*
Ethyl tetradecanoate, in volatile composition analysis of limoncello, 183*t*
Ethyl valerate, in volatile composition analysis of limoncello, 183*t*
2-Ethyl-3,5-dimethylpyrazine, component in coffee, 82*t*
2-Ethyl-3,5(6)-dimethylpyrazine, 81
2-Ethyl-3,6-dimethylpyrazine, component in coffee, 82*t*
4-Ethylguaiacol, component in coffee, 82*t*
2-Ethyl-4-hydroxy-5-methyl-3(2H)-furanone, component of Medium Roast Kenyan and Medium Roast Indo Robusta beans, 83, 86*t*
5-Ethyl-4-hydroxy-2-methyl-3(2H)-furanone, component of Medium Roast Kenyan and Medium Roast Indo Robusta beans, 83, 86*t*
3-Ethylpyridine, trigonelline breakdown derivatives, 87
4-Ethylpyridine, trigonelline breakdown derivatives, 87
Eucalyptol, 168*t*, 170*t*, 171*t*

F

Fenchol, in volatile composition analysis of limoncello, 183*t*, 189*t*
Fenchone, in volatile composition analysis of limoncello, 183*t*
Flavonoids, polyphenols in tea, 46
Flavor dilution factors, in tea aroma extracts, 27*t*, 28
Flavors, in non-carbonated beverages, overview of, 3
  alcoholic beverages, 17
  berry flavors, 12
  coffee, 8
  dairy beverages, 15
  flavored water, 15
  fruit juices/oils, 9, 12
  grape flavors, 13
  sports/energy drinks, 15
  tea, 4
Floral aroma, 96, 97*f*, 97*t*
Floral notes, 83
Florida orange five fold oil chromatogram, 10, 11*f*
Folate, 117, 119, 120
  ascorbic acid and 2-(mercapto)-ethanol for maximum stability of, 119

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ix002

  determination using stable isotpe dilution assay, 119
    structures of, 117, 118*f*
  5-formyltetrahydrofolate, 121
  5-methyltetrahydrofolate, 120
  stability
    effects of processing on, 120
    effects of storage on, 124
  tetrahydrofolate, 121
Folded fruit oils, 10
  Argentinian lemon single fold oil chromatogram, 10, 12*f*
  Californian grapefruit five fold oil chromatogram, 11, 13*f*
  Florida orange five fold oil chromatogram, 10, 11*f*
Folic acid, SIDA used folate vitamers and pantothenic acid, structures of, 117, 118*f*
10-Formylfolate, SIDA used folate vitamers and pantothenic acid, structures of, 117, 118*f*
5-Formyltetrahydrofolate, 121
  SIDA used folate vitamers and pantothenic acid, structures of, 117, 118*f*
Fruit juices, 9, 12
  apple, 13
  berry flavors, 12
    strawberry sweet charley liquid/liquid extract, 12, 14*f*
    volatile/non-volatile components of, 12
  beverages, consumer choices in selection of, 136
  cherry flavors, 13
  global growth by region from 2003 to 2008, 10, 11*t*
  grape flavors, 13
    4-methyl-4-mercaptopentan-2-one sulfur, 13, 14*f*
  pineapple juice, 14
    S-sinapyl glutathione peptide from, 14, 16*f*
  production processes, 130
    by conventional, 130
    by enzymatic release, 131
    by HHP, 131
    by hot pressing, 130
  sulfur compounds trace levels in, 13
  superfruits with health benefits and antioxidant properties, 15
  See also Blueberry juice
Fruit oils, 9
  cold press, 10
    Persian Lime cold press oil chromatogram, 11, 12*f*
  folded, 10
    Argentinian lemon single fold oil chromatogram, 10, 12*f*
    Californian grapefruit five fold oil chromatogram, 11, 13*f*
    Florida orange five fold oil chromatogram, 10, 11*f*
  non-volatiles, flavonoid glycoside, Rutin, 11, 13*f*
  volatiles
    grapefruit, 11, 13*f*
    lemon, 10, 12*f*
    lime, 11, 12*f*
    orange, 10, 11*f*
  washed, 10
Fruity aroma, 96, 97*f*, 97*t*
Functional groups, in Sri Lankan black teas
  spider charts, 26, 26*f*
  stacked bar graph, 24*f*, 26
Furaneol
  component in coffee, 82*t*
  compound formed during sugar degradation process, 83, 87*f*
Furfural
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 183*t*
Furfuryl alcohol, in lemon peel ethanolic extract volatile component analysis, 179*t*
Furfuryl mercaptan, component in coffee, 82*t*
Furfuryl methyl disulfide, component in coffee, 82*t*
Furfuryl methyl sulfide, component in coffee, 82*t*
1-(2-Furyl)-*(E)*-1-buten-3-one, component of Medium Roast

Kenyan and Medium Roast Indo Robusta beans, 83, 86*t*

1-(2-Furyl)-trans-1-buten-3-one, compound formed during sugar degradation process, 83, 87*f*

G

Gallic acid, relative contents *versus* price in Long Jin tea, 40, 41*f*

γ-Bisabolene i, in volatile composition analysis of limoncello, 183*t*

γ-Bisabolene ii, in volatile composition analysis of limoncello, 183*t*

γ-Decalactone enantiomers ratio, 28*t*, 29

γ-Muurolene, in volatile composition analysis of limoncello, 183*t*

γ-Nonalactone enantiomers ratio, 28*t*, 29

γ-Terpinene, in lemon peel ethanolic extract volatile component analysis, 179*t*

γ-Terpinene, in volatile composition analysis of limoncello, 183*t*, 189*t*

γ-Terpinyl acetate, in volatile composition analysis of limoncello, 183*t*

Gas chromatography analysis, on ethanolic extract, 182

Gas chromatography-mass spectrometry analysis, on ethanolic extract, 182

Gas chromatography-olfactometry analysis, on volatile components of limoncello, 182, 189*t*

Geosmin, negatively perceived contributor in Robusta coffee, 81, 84*f*

Geranial
- in lemon peel ethanolic extract volatile component analysis, 179*t*
- reaction from citral to potent off notes, 145*f*
- in volatile composition analysis of limoncello, 183*t*, 189*t*

Geranic acid, in volatile composition analysis of limoncello, 183*t*

Geraniol
- in lemon peel ethanolic extract volatile component analysis, 179*t*
- in volatile composition analysis of limoncello, 183*t*, 189*t*

Geranyl acetate
- in lemon peel ethanolic extract volatile component analysis, 179*t*
- in volatile composition analysis of limoncello, 183*t*, 189*t*

Geranyl acetone, in volatile composition analysis of limoncello, 183*t*

Geranyl propionate
- in lemon peel ethanolic extract volatile component analysis, 179*t*
- in volatile composition analysis of limoncello, 183*t*

Grape flavors, 13
- 4-methyl-4-mercaptopentan-2-one sulfur, 13, 14*f*

Green aroma, 96, 97*f*, 97*t*

Green tea, 46
- polyphenols in, 46, 48*f*

Guaiacol, component in coffee, 82*t*

Gyokuro Japanese green tea, liquid/liquid extract chromatogram, 6, 7*f*

H

Heptadecanal, in volatile composition analysis of limoncello, 183*t*

Heptanal, in lemon peel ethanolic extract volatile component analysis, 179*t*

Heptyl acetate, in volatile composition analysis of limoncello, 183*t*

Herbal notes, 83

Herbal tea, 4

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ix002

Herboxide. *See* 2-Methyl-5-isopropenyl-2-vinyltetrahydrofuran
Hesperetin, 63*f*, 65*t*, 67, 69, 69*t*
Hesperidin, 69, 69*t*, 70*f*
Hexadecanal, in volatile composition analysis of limoncello, 183*t*
Hexadecane, in volatile composition analysis of limoncello, 183*t*
Hexadecanoic acid, in volatile composition analysis of limoncello, 183*t*
Hexanal
  apple juice aroma component, 105, 106*t*, 109*t*, 111*t*, 112*t*
  in volatile composition analysis of limoncello, 183*t*
Hexanal odorant, 98*t*, 99
Hexanoic acid, in volatile composition analysis of limoncello, 183*t*, 189*t*
Hexanol
  apple juice aroma component, 105, 106*t*, 109*t*, 111*t*, 112*t*
  in volatile composition analysis of limoncello, 183*t*
Hexan-2-ol, in volatile composition analysis of limoncello, 183*t*
Hexyl acetate, apple juice aroma component, 105, 106*t*, 109*t*, 111*t*, 112*t*
High hydrostatic pressure
  process for fruit juice production, 131
  treatment effects on blueberry juice quality, 137, 138*f*
High performance liquid chromatography, 116
High-temperature-short-time treatment, 123
Homoeriodictyol, 63*f*, 65*t*, 67
Homofuraneol, compound formed during sugar degradation process, 83, 87*f*
Homofuronol, compound formed during sugar degradation process, 83, 87*f*
Honeybush tea extract. *See* *Cyclopia intermedia* extract
Hot pressing, for fruit juice production, 130
8-Hydroperoxy-p-cymene, reaction from citral to potent off notes, 145*f*
Hydrophilic exterior, cyclodextrin models showing geometry and physical dimensions of, 144, 146*f*
Hydrophobic core, cyclodextrin models geometry and physical dimensions of, 144, 146*f*
2-Hydroxy-β-santalene, in volatile composition analysis of limoncello, 183*t*
2-Hydroxy-2-cyclopentenone, in lemon peel ethanolic extract volatile component analysis, 179*t*
5-Hydroxy-5,6-dihydromaltol, in lemon peel ethanolic extract volatile component analysis, 179*t*
3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone, concentrations in Sri Lankan black teas, 29*f*, 30
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone, in Sri Lankan black teas
  calibration curves, 23*t*
  concentrations of, 29*f*, 30
4-Hydroxyhexan-2,3,5-trione, in lemon peel ethanolic extract volatile component analysis, 179*t*
2-Hydroxy-3-methyl-2-cyclopenten-1-one, component of Medium Roast Kenyan and Medium Roast Indo Robusta beans, 83, 86*t*
5-Hydroxymethylfurfural, in lemon peel ethanolic extract volatile component analysis, 179*t*
3-Hydroxy-2-methyl-4(4H)-pyranone, component of Medium Roast Kenyan and Medium Roast Indo Robusta beans, 83, 86*t*

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ix002

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ix002

I

isoAmyl acetate, in volatile composition analysis of limoncello, 183*t*
2-isoButyl-3-methoxypyrazine, component in coffee, 82*t*, 83
isoCitral, in volatile composition analysis of limoncello, 183*t*
isoPiperitenone, in volatile composition analysis of limoncello, 183*t*
Isoprene, in volatile composition analysis of limoncello, 183*t*
7-isoPropenylcoumarin, in volatile composition analysis of limoncello, 183*t*
2-isoPropyl-3-methoxypyrazine odorant, 98*t*, 99
isoPulegol i, in volatile composition analysis of limoncello, 183*t*
isoPulegol ii, in volatile composition analysis of limoncello, 183*t*
isoValeraldehyde, component in coffee, 82*t*
Isovaleric acid, in lemon peel ethanolic extract volatile component analysis, 179*t*
isoValeric acid, in volatile composition analysis of limoncello, 183*t*, 189*t*

K

Kandy Sri Lankan black teas
  AEDA results, 28, 29
  characteristic odorants of, 21
  chiral compound enantiomers, ratios of, 28*t*, 29
  FD factors of aroma compounds in, 27*t*, 28
  functional groups in
    spider charts, 26, 26*f*
    stacked bar graph, 24*f*, 26
  3HDMF concentrations in, 29*f*, 30
  4HDMF in
    calibration curves of, 23*t*
    concentrations of, 29*f*, 30
  sensory characteristics of, 25
Keemum tea, 37
  taste active compounds, 36*t*, 37
  taste profiles, 39, 40*f*
Kenyan Arabica Coffee. *See Coffea arabica*
Kenyan brew coffee
  compounds of, 84, 88*f*

L

LC Taste®
  for sensory evaluation of teas, 67
    Aspalathus linearis extract, 72, 72*t*
    Cyclopia intermedia extract, 68, 69*t*
    Eriodicyton angustifolium extract, 65*t*, 67
  for taste modulating compounds identification from teas, 61
    Aspalathus linearis extract, 71*f*, 72, 72*t*
    Cyclopia intermedia extract, 69, 69*t*, 70*f*
    Eriodicyton angustifolium extract, 63*f*, 65*t*, 67
Lemon emulsion systems
  with β-cyclodextrin sensory evaluation, 156*f*
  beverage systems composition, 148*t*, 150
Lemon peel, ethanolic extract volatile component analysis, 179*t*, 182
Limettin, in volatile composition analysis of limoncello, 183*t*
Limoncello liqueur
  chromatogram extract profile, 182, 188*f*
  volatile component extract analysis of, 177, 182, 183*t*, 189*t*
Limonen-1,2-diol, in volatile composition analysis of limoncello, 183*t*
Limonene
  in lemon peel ethanolic extract volatile component analysis, 179*t*

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ix002

in volatile composition analysis of limoncello, 183*t*, 189*t*
Limonene-1,2-epoxide i, in volatile composition analysis of limoncello, 183*t*
Limonene-1,2-epoxide ii, in volatile composition analysis of limoncello, 183*t*
Limonen-4-ol, in volatile composition analysis of limoncello, 183*t*, 189*t*
Limonen-10-ol, in volatile composition analysis of limoncello, 183*t*
Limonen-10-yl acetate
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 183*t*
Limonen-4-yl hydroperoxide, in volatile composition analysis of limoncello, 183*t*
Linalool
  component in coffee, 82*t*
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 183*t*, 189*t*
Linalool enantiomers ratio, in Sri Lankan black teas, 28*t*, 29
Linoleic acid, in volatile composition analysis of limoncello, 183*t*
Linoleic acid surface tension, 172, 172*t*
Linolenic acid, in volatile composition analysis of limoncello, 183*t*
Lipton tea, 37
  taste active compounds, 36*t*, 37
  taste profiles, 39, 40*f*
Long Jin tea, 37
  ^{1}H NMR spectrum, 40, 41*f*
    relative ratio of eight taste active compounds from, 40, 41*f*
  price *versus* relative contents of gallic acid in, 40, 41*f*
  taste active compounds, 36*t*, 37
  taste profiles, 39, 40*f*

M

Maltol
  component in coffee, 82*t*
  compound formed during sugar degradation process, 83, 87*f*
Mangiferin, 69*t*, 70*f*
Mass spectrometry, using stable isotope dilution assay, 116
Medium Roast Kenyan bean, 83, 84*f*, 86*f*
  comparison of roasting temperatures for, 86, 87, 88*f*, 89*t*
  concentration of trigonelline roasting temperatures, 87, 89*t*
Medium Roast Robusta bean, 83, 86*f*
Megastigmatrienone. *See* 3,5,5-Trimethyl-4-(2-butenylidene)-2-cyclohexenone
Melonal, in volatile composition analysis of limoncello, 183*t*, 189*t*
2-Me-6-methylene-1,7-octadien-3-ol, in volatile composition analysis of limoncello, 183*t*
p-Menthadien-ol(s), SPME analysis of, 148*f*, 151
p-Menthadien-8-ols, reaction from citral to potent off notes, 145*f*
2-(Mercapto)-ethanol, for maximum stability of folate, 119
Methional, component in coffee, 82*t*
2-Methoxy-4-vinylphenol
  component in coffee, 82*t*
  in lemon peel ethanolic extract volatile component analysis, 179*t*
Methyl geranate, in volatile composition analysis of limoncello, 183*t*
p-Methylacetophenone
  formation SPME results, 153*f*
  reaction from citral to potent off notes, 145*f*
6-Methylbicyclo[3.3.0]oct-2-en-7-one, in volatile composition analysis of limoncello, 183*t*

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ix002

2-Methylbutanal, component in coffee, 82*t*
2-Methylbutanol, apple juice aroma component, 105, 106*t*, 109*t*, 111*t*, 112*t*
2-Methylbutan-2-ol, in volatile composition analysis of limoncello, 183*t*
3-Methylbutan-2-one, in volatile composition analysis of limoncello, 183*t*
3-Methyl-1-butene, in volatile composition analysis of limoncello, 183*t*
3-Methyl-3-butenoic acid, in lemon peel ethanolic extract volatile component analysis, 179*t*
2-Methylbutyl acetate
  apple juice aroma component, 105, 106*t*, 109*t*, 111*t*, 112*t*
  in volatile composition analysis of limoncello, 183*t*
1-Methyl-1,3-cyclohexadiene, in volatile composition analysis of limoncello, 183*t*
3-Methyl-3-cyclohexenone, in volatile composition analysis of limoncello, 183*t*
5-Methylcyclopentapyrazine, component in coffee, 82*t*
5-Methyl-6,7-dihydro-5H-cylcopentapyrazine, 81
6-Methyl-5-hepten-2-one
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 183*t*
2-Methylisoborneol, negatively perceived contributor in Robusta coffee, 81, 84*f*
2-Methyl-5-isopropenyl-2-vinyltetrahydrofuran, compound of Kenyan brew coffee, 84, 88*f*
Methylnicotinate, trigonelline breakdown derivatives, 87
2-Methyl-2-propenal, in volatile composition analysis of limoncello, 183*t*
2-Methylpyridine, trigonelline breakdown derivatives, 87
3-Methylpyridine, trigonelline breakdown derivatives, 87
5-Methyltetrahydrofolate, 120
  SIDA used folate vitamers and pantothenic acid, structures of, 117, 118*f*
3-(Methylthio)-propanal odorant, 98*t*, 99
Model wine surface tension, 172, 172*t*
Monoterpene ethyl ether, in volatile composition analysis of limoncello, 183*t*
Muscadine grapes, processing effects on, 138*f*, 139
Myrtenol, in volatile composition analysis of limoncello, 183*t*

N

Nantou Oolong tea, liquid/liquid extract chromatogram, 6, 7*f*
Neral
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  reaction from citral to potent off notes, 145*f*
  in volatile composition analysis of limoncello, 183*t*, 189*t*
Nerol
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 183*t*, 189*t*
Neryl acetate
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 183*t*, 189*t*
NFC juices, aroma index model application, 106, 108
Nicotinic acid, trigonelline breakdown derivatives, 87
Nonanal, in volatile composition analysis of limoncello, 183*t*

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ix002

Nonanal diethyl acetal, in volatile composition analysis of limoncello, 183*t*
Nonanoic acid, in volatile composition analysis of limoncello, 183*t*
Nonanol, in volatile composition analysis of limoncello, 183*t*
Non-volatiles, in teas, study of, 33
  analytical results of, 37
Nonyl acetate
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 183*t*
Nootkatone, in volatile composition analysis of limoncello, 183*t*
Nothofagin, 71*f*, 72*t*
Nuclear magnetic resonance
  Long Jin green tea
    ^{1}H spectrum of, 40, 41*f*
    relative ratio of eight taste active compounds, 40, 41*f*
  for tea quality assessment, 40
Nuwara Eliya Sri Lankan black teas
  AEDA results, 28
  characteristic odorants of, 21
  chiral compound enantiomers, ratios of, 28*t*, 29
  FD factors of aroma compounds in, 27*t*, 28
  functional groups in
    spider charts, 26, 26*f*
    stacked bar graph, 24*f*, 26
  3HDMF concentrations in, 29*f*, 30
  4HDMF in
    calibration curves of, 23*t*
    concentrations of, 29*f*, 30
  sensory characteristics of, 25

O

Octanal
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 183*t*, 189*t*
Octanal diethyl acetal, in volatile composition analysis of limoncello, 183*t*
Octane, in volatile composition analysis of limoncello, 183*t*
Octanoic acid, in volatile composition analysis of limoncello, 183*t*, 189*t*
Octanol, in volatile composition analysis of limoncello, 183*t*
1-Octen-3-one, 167, 168*t*, 169*f*, 170*f*, 170*t*, 171*t*
1-Octen-3-one odorant, 98*t*, 99
Octyl acetate, in volatile composition analysis of limoncello, 183*t*
Odorants, of coffee, 77, 82*t*
Oleic acid, in volatile composition analysis of limoncello, 183*t*
o-Aminoacetophenone odorant, 98*t*, 99
Oolong tea, 46
Organic acids, taste active compounds in tea, 36*t*, 37
Organoleptic
  properties of odor thresholds in water
    p,α-dimethylstyrene, 146*t*
    p-cresol, 146*t*
    p-cymene, 146*t*
    p-cymen-8-ol, 146*t*
    p-methylacetophenone, 146*t*
  reaction from citral to potent off notes, 145*f*
Ortho-dihydroxy, 47
Oxidation, reaction from citral to potent off notes, 145*f*
Oxygen radical absorbance capacity, of blueberry juice, 134, 136*f*

P

p,α-Dimethylstyrene
  in volatile composition analysis of limoncello, 183*t*, 189*t*
  organoleptic property of odor thresholds in water, 146*t*
  for SPME results, 151*f*, 153*f*
Pantoic acid, lactonization of, 117, 120*f*

Pantothenic acid, 117, 123
  acidic hydrolysis of, 117, 120*f*
  as derivative of pantoic acid, 117
  stability
    effects of processing on, 120
    effects of storage on, 124
  using stable isotope dilution assay determination of, 119
p-Cymene
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 183*t*, 189*t*
p-Cymen-8-ol, in volatile composition analysis of limoncello, 183*t*, 189*t*
Pentadecanal, in volatile composition analysis of limoncello, 183*t*
Pentadecanoic acid, in volatile composition analysis of limoncello, 183*t*
Pentan-2,3-dione, component in coffee, 82*t*
Pentan-2-ol, in volatile composition analysis of limoncello, 183*t*
Perilla aldehyde, in volatile composition analysis of limoncello, 183*t*
Persian lime cold press oil chromatogram, 11, 12*f*
Phenolic acids, in tea polyphenols, 46
Phenylacetaldehyde
  component in coffee, 82*t*
  in volatile composition analysis of limoncello, 183*t*
Pinanol, in volatile composition analysis of limoncello, 183*t*
Pineapple juice, 14
  S-sinapyl glutathione peptide from, 14, 16*f*
Piperitone, in volatile composition analysis of limoncello, 183*t*
p-Mentha-1,5,8-triene, in volatile composition analysis of limoncello, 183*t*
p-Menth-1-en-9-yl acetate, in volatile composition analysis of limoncello, 183*t*
p-Menth-2-enyl ethyl ether, in volatile composition analysis of limoncello, 183*t*, 189*t*
Polyphenolics, taste active compounds in tea, 36*t*, 37
Polyphenols
  in alcoholic beverages, 17
  in berry flavors, 12
  in black tea, 45, 46
    astringency and bitterness of catechins and theaflavins for, 50, 52*t*
    bio-function properties, 50
    flavonoids, 46
    phenolic acids, 46
  in green tea, 46, 48*f*
  proposed mechanism of theaflavin formation, 47, 49*f*
  structure of theadibenzotropolone A, 49, 51*f*
  in tea, 5, 6
Pomegranate juice. *See Punica granatum L.* juice
Prenol, in volatile composition analysis of limoncello, 183*t*
Propanal, 168*t*, 169*f*
Propionic acid, component in coffee, 82*t*
pseudo-Limonene, in volatile composition analysis of limoncello, 183*t*
Punica granatum L. juice
  aroma components, 93
    Earthy, 96, 97*f*, 97*t*
    Floral, 96, 97*f*, 97*t*
    Fruity, 96, 97*f*, 97*t*
    Green, 96, 97*f*, 97*t*
  composition and sensory characteristics, 96, 97*f*, 97*t*
  odorants in, 96, 97, 98*t*
  taste/mouthfeel attributes
    astringent, 96, 97*f*, 97*t*
    sour, 96, 97*f*, 97*t*
    sweet, 96, 97*f*, 97*t*
Pyrazine, comparison of, 81, 85*f*
Pyridine, trigonelline breakdown derivatives, 87

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ix002

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ix002

R

Red wine, 17
  resveratrol structure in, 17, 17*f*
Resveratrol structure, 17, 17*f*
Robusta Coffee. *See Coffea canephora*
Rooibos tea extract. *See* Aspalathus linearis extract
Rosefuran, in volatile composition analysis of limoncello, 183*t*
Ruhuna Sri Lankan black teas
  AEDA results, 28, 29
  characteristic odorants of, 21
  chiral compound enantiomers, ratios of, 28*t*, 29
  FD factors of aroma compounds in, 27*t*, 28
  functional groups in
    spider charts, 26, 26*f*
    stacked bar graph, 24*f*, 26
  3HDMF concentrations in, 29*f*, 30
  4HDMF in
    calibration curves of, 23*t*
    concentrations of, 29*f*, 30
  sensory characteristics of, 25
Rutin flavonoid glycoside, 11, 13*f*

S

Sabinene, in volatile composition analysis of limoncello, 183*t*, 189*t*
Sabinol, in volatile composition analysis of limoncello, 183*t*
Salty, taste thresholds data in teas, 38*t*, 39
Selina-3,11-diene, in volatile composition analysis of limoncello, 183*t*
Sesquisabinene hydrate, in volatile composition analysis of limoncello, 183*t*
Sesquiterpene oxygenated compound, in volatile composition analysis of limoncello, 183*t*
Sotolone, compound formed during sugar degradation process, 83, 87*f*
Sour, taste thresholds data in teas, 38*t*, 39
Sports/energy drinks, 15
  global consumption by category from 2003 to 2008, 15, 16*t*
  key non-volatile constituents of, 15, 16*f*
Spring tea, 37
  taste active compounds, 36*t*, 37
  taste profiles, 39, 40*f*
Squalene, in volatile composition analysis of limoncello, 183*t*
Sri Lankan black teas
  Dimbula
    AEDA results, 28, 29
    characteristic odorants of, 21
    chiral compound enantiomers, ratios of, 28*t*, 29
    FD factors of aroma compounds in, 27*t*, 28
    functional groups bar graph and spider charts in, 24*f*, 26, 26*f*
    3HDMF concentrations in, 29*f*, 30
    4HDMF in, 23*t*, 29*f*, 30
    sensory characteristics of, 25
  Kandy
    AEDA results, 28, 29
    characteristic odorants of, 21
    chiral compound enantiomers, ratios of, 28*t*, 29
    FD factors of aroma compounds in, 27*t*, 28
    functional groups bar graph and spider charts in, 24*f*, 26, 26*f*
    3HDMF concentrations in, 29*f*, 30
    4HDMF in, 23*t*, 29*f*, 30
    sensory characteristics of, 25
  Nuwara Eliya
    AEDA results, 28
    characteristic odorants of, 21
    chiral compound enantiomers, ratios of, 28*t*, 29
    FD factors of aroma compounds in, 27*t*, 28
    functional groups bar graph and spider charts in, 24*f*, 26, 26*f*

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ix002

        3HDMF concentrations in, 29*f*, 30
        4HDMF in, 23*t*, 29*f*, 30
        sensory characteristics of, 25
    Ruhuna
        AEDA results, 28, 29
        characteristic odorants of, 21
        chiral compound enantiomers, ratios of, 28*t*, 29
        FD factors of aroma compounds in, 27*t*, 28
        functional groups bar graph and spider charts in, 24*f*, 26, 26*f*
        3HDMF concentrations in, 29*f*, 30
        4HDMF in, 23*t*, 29*f*, 30
        sensory characteristics of, 25
    Uva
        AEDA results, 28
        characteristic odorants of, 21
        chiral compound enantiomers, ratios of, 28*t*, 29
        FD factors of aroma compounds in, 27*t*, 28
        functional groups bar graph and spider charts in, 24*f*, 26, 26*f*
        3HDMF concentrations in, 29*f*, 30
        4HDMF in, 23*t*, 29*f*, 30
        sensory characteristics of, 25
S-sinapyl glutathione peptide, from pineapple, 14, 16*f*
Stable isotope dilution assay
    determination of folate and
        pantothenic acid using, 119
        structures of, 117, 118*f*
    mass spectrometry using, 116
Sterubin, 63*f*, 65*t*
Strawberries, processing effects on, 139
    anthocyanin content, 139
Strawberry sweet charley, liquid/liquid extract, 12, 14*f*
Styrene, in volatile composition analysis of limoncello, 183*t*
Sugars, taste active compounds in tea, 36*t*, 37
Sulfur compounds, trace levels in fruit juices, 13
Sum of esters, correlation in commercial apple juices from concentrate, 111, 112*f*
Surface tension, 172
    of BSA, 172, 172*t*
    of ethanol, 172, 172*t*
    of linoleic acid, 172, 172*t*
    of model wine, 172, 172*t*
    of water, 172, 172*t*
Sweet, taste thresholds data in teas, 38*t*, 39

T

Taste, bio-function properties of polyphenols in black tea, 50
Taste modulating compounds
    identification from teas using LC
        Taste®, 61
        Aspalathus linearis extract, 71*f*, 72, 72*t*
        Cyclopia intermedia extract, 69, 69*t*, 70*f*
        Eriodicyton angustifolium extract, 63*f*, 65*t*, 67
t-Cadinol, in volatile composition analysis of limoncello, 183*t*
Tea, 4
    amino acid related analogues in, 35*f*
    chlorogenic acid in, 7, 8*f*
        and its analogues, 8*f*
    differentiation of, 39
    herbal, 4
    Keemum tea, 37
        taste profiles, 39, 40*f*
    Lipton tea, 37
        taste profiles, 39, 40*f*
    Long Jin tea, 37
        ^{1}H NMR spectrum, 40, 41*f*
        price *versus* relative contents of gallic acid in, 40, 41*f*
        taste profiles, 39, 40*f*
    non-volatiles in, study of, 33
        analytical results of, 36*t*, 37, 39
    producers, 4
        China, 4
        India, 4
        Kenya, 4

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ix002

- Sri Lanka, 4
- quality assessment by NMR, 40
  - ^{1}H spectrum of Long Jin green tea, 40, 41*f*
- RTD, global consumption by category from 2003 to 2008, 4, 6*t*
- sensory evaluation via LC Taste®, 67
  - *Aspalathus linearis* extract, 72, 72*t*
  - *Cyclopia intermedia* extract, 68, 69*t*
  - *Eriodicyton angustifolium* extract, 65*t*, 67
- Spring tea, 37
  - taste profiles, 39, 40*f*
- taste active compounds, 36*t*, 37
  - amino acid, 36*t*, 37
  - cations, 36*t*, 37
  - organic acids, 36*t*, 37
  - polyphenolics, 36*t*, 37
  - sugars, 36*t*, 37
- taste modulating compounds identification using LC Taste®, 61
  - *Aspalathus linearis* extract, 71*f*, 72, 72*t*
  - *Cyclopia intermedia* extract, 69, 69*t*, 70*f*
  - *Eriodicyton angustifolium* extract, 63*f*, 65*t*, 67
- taste thresholds data, 38*t*, 39
  - astringent, 38*t*, 39
  - bitter, 38*t*, 39
  - salty, 38*t*, 39
  - sour, 38*t*, 39
  - sweet, 38*t*, 39
  - umami, 38*t*, 39
- types of, 4
  - Black teas, 4
  - *Camellia sinensis*, 4
  - Green tea, 4
  - Oolong teas, 4
  - *Sinensis assamica*, 4
  - *Sinensis sinensis*, 4
  - White tea, 4
- volatile/non-volatile components of, 5
  - caffeine, 5, 7*f*
  - Gyokuro Japanese Green tea, liquid/liquid extract chromatogram, 6, 7*f*
  - Nantou Oolong tea, liquid/liquid extract chromatogram, 6, 7*f*
  - polyphenols, 5, 6

Terpinen-4-ol
- in lemon peel ethanolic extract volatile component analysis, 179*t*
- in volatile composition analysis of limoncello, 183*t*, 189*t*

Terpinen-4-yl ethyl ether, in volatile composition analysis of limoncello, 183*t*, 189*t*

Terpinolene, in lemon peel ethanolic extract volatile component analysis, 179*t*

Terpinolene, in volatile composition analysis of limoncello, 183*t*, 189*t*

Tetradecane, in volatile composition analysis of limoncello, 183*t*

Tetradecanoic acid, in volatile composition analysis of limoncello, 183*t*

1-Tetradecene, in volatile composition analysis of limoncello, 183*t*

Tetrahydrofolate, 121
- SIDA used folate vitamers and pantothenic acid, structures of, 117, 118*f*

Theadibenzotropolone A structure, 49, 51*f*

Theaflavin-3,3’-digallate structure, 47, 48*f*

Theaflavin-3-gallate structure, 47, 48*f*

Theaflavin-3’-gallates structure, 47, 48*f*

Theaflavins
- astringency and bitterness in black tea, 50, 52*t*
- by enzymatic co-oxidation of catechins
  - *ortho*-dihydroxy, 47
  - *vic*-trihydroxy, 47
- formation proposed mechanism of, 47, 49*f*
- structure of, 47, 48*f*

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ix002

    structures of
        theaflavin, 47, 48*f*
        theaflavin-3,3’-digallate, 47, 48*f*
        theaflavin-3-gallate, 47, 48*f*
        theaflavin-3’-gallates, 47, 48*f*
Thearubigins, as heterogeneous mixture of pigments, 47
2,4(10)-Thujadiene, in volatile composition analysis of limoncello, 183*t*
Thymol, in volatile composition analysis of limoncello, 183*t*
Toluene, in volatile composition analysis of limoncello, 183*t*
Total phenolic content, of blueberry juice, 134, 135*f*
2,4,6-Trichloroanisole, negatively perceived contributor in Robusta coffee, 81, 84*f*
Tricosane, in volatile composition analysis of limoncello, 183*t*
Tricyclene, in volatile composition analysis of limoncello, 183*t*
Tridecanal, in volatile composition analysis of limoncello, 183*t*
Tridecane, in volatile composition analysis of limoncello, 183*t*
Trigonelline
    breakdown derivatives
        3-ethylpyridine, 87
        4-ethylpyridine, 87
        methylnicotinate, 87
        2-methylpyridine, 87
        3-methylpyridine, 87
        nicotinic acid, 87
        pyridine, 87
    roasting temperatures of Kenyan bean, 87, 89*t*
3,5,5-Trimethyl-4-(2-butenylidene)-2-cyclohexenone, compound of Kenyan brew coffee, 84, 88*f*
Trimethylpyrazine, component in coffee, 81, 82*t*

U

Umami, taste thresholds data in teas, 38*t*, 39
Undecanal
    in lemon peel ethanolic extract volatile component analysis, 179*t*
    in volatile composition analysis of limoncello, 183*t*
Undecanal diethyl acetal, in volatile composition analysis of limoncello, 183*t*
Undecane, in volatile composition analysis of limoncello, 183*t*
Uva Sri Lankan black teas
    AEDA results, 28
    characteristic odorants of, 21
    chiral compound enantiomers, ratios of, 28*t*, 29
    FD factors of aroma compounds in, 27*t*, 28
    functional groups in
        spider charts, 26, 26*f*
        stacked bar graph, 24*f*, 26
    3HDMF concentrations in, 29*f*, 30
    4HDMF in
        calibration curves of, 23*t*
        concentrations of, 29*f*, 30
    sensory characteristics of, 25

V

Valencene
    in lemon peel ethanolic extract volatile component analysis, 179*t*
    in volatile composition analysis of limoncello, 183*t*, 189*t*
Valeraldehyde, in volatile composition analysis of limoncello, 183*t*, 189*t*
Valeraldehyde diethyl acetal, in volatile composition analysis of limoncello, 183*t*
Vanillin, in volatile composition analysis of limoncello, 183*t*
Verbenone, in volatile composition analysis of limoncello, 183*t*
Vic-trihydroxy, 47
4-Vinylphenol, in lemon peel ethanolic extract volatile component analysis, 179*t*

Downloaded by 89.163.35.42 on June 21, 2012 | http://pubs.acs.org
Publication Date (Web): March 18, 2010 | doi: 10.1021/bk-2010-1036.ix002

Volatiles
  fresh and aged limoncello composition, 182, 183*t*, 189*t*
  in limoncello liqueur analysis, 177

W

Washed fruit oils, 10
Water, flavored, 5*t*, 15
Water-ethanol solution system
  odorant release, 166
  and bulk phase mass transfer mechanism, 167, 168, 169
  comparison under DHS conditions, 166
    ethyl 2-butenoate, 168*t*, 170, 170*t*, 171*f*, 171*t*
    ethyl butyrate, 168*t*, 169*f*
    eucalyptol, 168*t*, 170*t*, 171*t*
    1-octen-3-one, 167, 168*t*, 169*f*, 170*f*, 170*t*, 171*t*
    propanal, 168*t*, 169*f*
  effect of air-water partition coefficient on, 166
    under DHS conditions, 166, 167*f*
    HS concentrations relative change *versus* log P values, 164*f*, 166
  as function of ethanol content, 167, 169*f*
  surface tension, 172, 172*t*
Winey/berry, comparison of, 82, 85*f*

Y

Yerba Santa extract. *See Eriodicyton angustifolium* extract

Z

(Z)-α-Bergamotene
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 183*t*
(Z)-α-Bisabolene
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 183*t*
(Z)-β-Ocimene
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 189*t*
(Z)-β-Ocimene, in volatile composition analysis of limoncello, 183*t*
(Z)-4,8-Dimethyl-1,3,7-nonatriene, in volatile composition analysis of limoncello, 183*t*
(Z)-3-Hexenal odorant, 98*t*, 99
(Z)-3-Hexenol, in volatile composition analysis of limoncello, 183*t*, 189*t*
(Z)-3-Hexenol odorant, 98*t*, 99
(Z)-Piperitol, in volatile composition analysis of limoncello, 183*t*
(Z)-p-Mentha-1(7),8-dien-2-ol, in volatile composition analysis of limoncello, 183*t*
(Z)-p-Mentha-2,8-dienol, in volatile composition analysis of limoncello, 183*t*, 189*t*
(Z)-p-Menth-2-enol, in volatile composition analysis of limoncello, 183*t*
(Z)-Sabinene hydrate
  in lemon peel ethanolic extract volatile component analysis, 179*t*
  in volatile composition analysis of limoncello, 183*t*
(Z)-Sabinene hydrate et ether, in volatile composition analysis of limoncello, 189*t*
(Z)-Sabinene hydrate ethyl ether, in volatile composition analysis of limoncello, 183*t*